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Biodegradation treatment of petrochemical wastewaters

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Resumo

O petróleo é um dos elementos mais relevantes para a economia mundial, pelo que o aumento do consumo de petróleo a nível mundial tem crescido ao longo dos anos. A produção mundial de petróleo é superior a três biliões de toneladas por ano, e os stocks de petróleo são significativos em muitas regiões do globo. O petróleo não só é uma fonte de energia, mas também é usado na produção de muitos químicos, como plásticos e cosméticos. A exploração das reservas de petróleo, a transformação do petróleo nas refinarias, os derrames e as águas resultantes da limpeza de reservatórios cria volumes elevadíssimos de efluentes que, devido à grande produção mundial de petróleo, estão sempre a aumentar. Estas águas residuais, com elevadas concentrações de poluentes, necessitam de um tratamento, usualmente complexo e caro. Estes factos originaram um interesse crescente pelo estudo de efluentes contaminados da indústria petroquímica assim como dos possíveis processos de tratamento (biológicos e químicos).

Um dos grupos de poluentes mais tóxicos que tem sido detectado nos efluentes de refinaria são os hidrocarbonetos de petróleo, nomeadamente alifáticos e aromáticos. Os hidrocarbonetos aromáticos policíclicos (PAHs) são dos componentes que merecem mais destaque e preocupação, devido à sua toxicidade (nomeadamente carcinogenicidade), persistência ambiental e resistência à degradação microbiana. Os PAHs de elevada massa molecular tendem a adsorver aos sedimentos, o que diminui o seu impacto ambiental, enquanto que os PAHs de baixa massa molecular se dissolvem mais facilmente em água, sendo transportados para as águas subterrâneas ou de superfície, tendo assim um maior impacto ambiental. De entre os 16 PAHs que fazem parte da lista de poluentes considerados prioritários pela Agência de Protecção Ambiental dos Estados Unidos (USEPA), foi seleccionado para este estudo o acenafteno, cujas fontes principais são as refinarias de petróleo e a queima do carvão.

A biodegradação apresenta vantagens do ponto de vista económico e ambiental em relação aos processos convencionais frequentemente usados para remoção de PAHs. Devido à presença destes compostos no meio ambiente, os microrganismos desenvolveram vias metabólicas para poder removê-los. Os microrganismos que biodegradam estes compostos na presença de oxigénio (aerobiose) são conhecidos desde o início do século XX, tendo sido feitos muitos estudos de biodegradação de PAHs em condições de aerobiose. No final dos anos 80, foram caracterizados microrganismos capazes de degradar hidrocarbonetos em condições de anaerobiose, e estudos posteriores demonstraram que estes microrganismos realizam degradação por vias metabólicas totalmente diferentes das usadas no metabolismo aeróbio. No entanto, a biodegradação em condições de anaerobiose está ainda menos explorada e o seu estudo poderá ser importante, uma vez que não há oxigénio disponível em todos os ambientes onde os PAHs estão presentes (por exemplo em sedimentos a grandes profundidades e em reservatórios de petróleo).

O objectivo deste estudo é a identificação de um consórcio de bactérias nativas do efluente da refinaria da GALP, localizada em Sines, com resistência toxicológica e capacidade de biodegradação de acenafteno em condições de anaerobiose. Para tal, foram formulados e

testados diferentes meios de cultura ricos em nutrientes, sais minerais, aceitadores de electrões e fontes de carbono diferentes (glucose e lactato), de modo a promover o máximo crescimento anaeróbio das bactérias presentes neste efluente. Foram seguidas duas estratégias de enriquecimento da comunidade microbiana. Numa das abordagens, o inóculo centrifugado foi adicionado ao meio de cultura num reactor, enquanto que na outra abordagem igual quantidade do efluente de refinaria e meio de cultura foram combinados no mesmo reactor. Em cada estratégia, a fonte de carbono usada foi diferente: glucose ou lactato. Em todos os reactores foi adicionado acenafteno a uma concentração de 100 µg/L. Ao longo do enriquecimento o crescimento microbiano foi seguido por medição da densidade óptica a 600 nm, enquanto que a monitorização da concentração de acenafteno foi efectuada por cromatografia em fase líquida. Foi também realizada a sequenciação do RNA ribossomal 16S para compreender a dinâmica das comunidades microbianas durante o enriquecimento nos diferentes reactores. A comparação dos diferentes reactores permitiu compreender que o crescimento da comunidade microbiana não depende da estratégia de inoculação seguida. O lactato foi seleccionado como a fonte de carbono a usar nos ensaios de resistência e biodegradação subsequentes por promover maior crescimento e biodiversidade da população microbiana. A comunidade obtida tinha como phyla maioritários *Proteobacteria* (68%) e *Firmicutes* (31%), enquanto a minoria era constituída por *Actinobacteria* (0.3%), *Synergistetes* (0.003%), *Thermotogae* (0.002%) e *Deinococcus-Thermus* (0.002%). Em termos de classes, o phylum *Proteobacteria* apresentou com predominância as seguintes classes: *Betaproteobacteria* (56%), *Alphaproteobacteria* (10%), *Proteobacteria* não classificadas (1%) e *Gamaproteobacteria* (0.5%). Por sua vez, o phylum *Firmicutes* teve como classe maioritária *Clostridia* (28%) e como membros menos representados *Firmicutes* não classificados (3%) e *Bacilli* (0.009%). Os phyla minoritários foram representados pelas classes *Actinobacteria* (0.3%), *Synergistia* (0.003%), *Thermotogae* (0.002%) e *Deinococci* (0.002%).

Para testar a toxicidade do acenafteno para a comunidade bacteriana seleccionada após o enriquecimento, assim como a sua estabilidade, foram realizados testes de resistência, onde se testaram diferentes concentrações de acenafteno (100-1500 µg/L). Os reactores não inoculados foram usados como controlos de adsorção, para testar a estabilidade do acenafteno. Tal como no ensaio anterior, o crescimento da comunidade foi acompanhado por medição da densidade óptica a 600 nm e a concentração de acenafteno foi monitorizada por cromatografia em fase líquida. Estes testes sugeriram que a concentração mais adequada para realizar os ensaios de biodegradação é 100 µg/L.

Os ensaios de biodegradação tiveram como objectivo avaliar o potencial de remoção do acenafteno pela comunidade microbiana na ausência e na presença de lactato, de forma a compreender se o acenafteno poderia ser usado como fonte de carbono única pelas bactérias ou se a presença de lactato seria necessária. Tal como no ensaio anterior, o crescimento da comunidade e a concentração de acenafteno foram monitorizadas. Para caracterizar e comparar as comunidades microbianas na presença e ausência de lactato, no início e no final do ensaio de biodegradação nos dois reactores, foi também efectuada sequenciação do RNA ribossomal 16S. Verificou-se que o lactato é necessário para o crescimento da população microbiana e que a remoção do composto é maior na sua presença, sugerindo que a

existência de uma fonte de carbono extra é necessária para a remoção do composto pela comunidade microbiana seleccionada após o enriquecimento. Na ausência de lactato obteve-se maioritariamente *Proteobacteria* (79%) e *Firmicutes* (15%), sendo que o phylum *Proteobacteria* teve como classes predominantes *Gamaproteobacteria* (46%) e *Betaprotebacteria* (27%), enquanto o phylum *Firmicutes* foi principalmente representado por *Clostridia* (14%) e *Bacilli* (0.08%). Na presença de lactato os phyla maioritários foram os mesmos, sendo que *Proteobacteria* foi ligeiramente mais abundante (84%) e *Firmicutes* ligeiramente menos abundante (6.4%). A principal diferença foi que a classe *Betaproteobacteria* passou a ser maioritária (66%), seguido da classe *Alphaproteobacteria* (14%) e por último *Gamaproteobacteria* (0.04%), enquanto o phylum *Firmicutes* foi principalmente representado por *Clostridia* (3.2%) e *Bacilli* (0.5%).

A instabilidade do acenafteno verificada através da sua remoção na ausência da biomassa em todos os ensaios realizados sugere que a sua remoção na presença da biomassa resultou não só de uma acção biológica, mas provavelmente também da adsorção ao vidro, o que poderá estar relacionado com a instabilidade que caracteriza os hidrocarbonetos policíclicos aromáticos de baixa massa molecular e a sua hidrofobicidade. Os resultados obtidos e descritos nesta tese indicam que o acenafteno é um composto instável e que a sua remoção por biodegradação só será uma vantagem se os tempos de residência nas estações de tratamento para os processos biológicos forem curtos.

Palavras chave: acenafteno; águas de refinaria; tratamento; caracterização de populações microbianas; biodegradação anaeróbia

Abstract

The demand for petroleum is always increasing. Therefore, refineries also face an increasing problem: to treat large volumes of oily wastewater containing hazardous compounds such as polycyclic aromatic hydrocarbons (PAHs). Acenaphthene, a polycyclic aromatic hydrocarbon, is among the 16 PAHs considered priority by the United States Environmental Protection Agency due to its environmental persistence and toxicity. Biodegradation treatment offers advantages in terms of environmental protection and costs over conventional treatments to remove PAHs from these oily wastewaters. Biodegradation of PAHs is not only possible under aerobic conditions, but also under anaerobic conditions. Oxygen is not present in all the environments containing PAHs and there are not a lot of studies addressing the biodegradation of acenaphthene under anaerobic conditions by microbial communities from refinery wastewaters. Therefore, further assessment in terms of the removal of this compound was carried out in the scope of the present thesis.

The aim of this thesis was to identify a consortium of bacteria, from a refinery wastewater, that could be able to remove acenaphthene under anaerobic conditions.

The refinery wastewater used to enrich the microbial community was obtained from the GALP refinery, located in Sines. Two different approaches were followed during the enrichment in terms of inoculation and carbon source (lactate and glucose). Since the microbial community growing in the presence of lactate presented higher growth and diversity, it was further addressed in resistance and biodegradation assays. The most abundant phyla of the community obtained were *Proteobacteria* (68%) and *Firmicutes* (31%). *Betaproteobacteria* (56%) and *Alphaproteobacteria* (10%) were the classes most represented of phylum *Proteobacteria*, whereas *Clostridia* (28%) was the most abundant class of phylum *Firmicutes*.

Resistance assays were carried out to assess the toxicity of acenaphthene to the microbial community as well as its stability by spiking acenaphthene in the reactors at different concentrations (100-1500 µg/L). This assay showed that the compound has less toxicity for the community at the lowest concentrations and presents some instability. Based on these results, it was decided to carry out subsequent biodegradation assays using acenaphthene at 100 µg/L. The biodegradation assay was performed to assess the ability of the microbial community to degrade acenaphthene with and without lactate as an additional carbon source. It was observed that acenaphthene is mainly removed in the presence of lactate and that the taxonomic profile of the microbial community is different depending on the presence of lactate. In its absence, *Proteobacteria* (79%) and *Firmicutes* (15%) were the most abundant phyla. The major classes of phylum *Proteobacteria* were *Gammaproteobacteria* (46%) and *Betaprotebacteria* (27%), whereas phylum *Firmicutes* was mainly represented by *Clostridia* (14%) and *Bacilli* (0.08%). In the presence of lactate, the most abundant phyla were similar, although *Proteobacteria* were slightly more abundant (84%) and *Firmicutes* were slightly less abundant (6.4%). The main difference was that *Betaproteobacteria* became the most abundant class (66%), followed by *Alphaproteobacteria* (14%) and *Gammaproteobacteria* (0.04%), whereas phylum *Firmicutes* was mainly represented by *Clostridia* (3.2%) and *Bacilli* (0.5%).

The instability of acenaphthene observed through its removal in the absence of bacteria in all the assays suggests that acenaphthene removal is not only due to bacterial metabolism, but probably also due to its adsorption to the glass of reactors, which can be related with the instability and hydrophobicity of polycyclic aromatic hydrocarbons (PAHs) of low molecular weight like acenaphthene. The results obtained and described in this thesis allow concluding that acenaphthene is an unstable compound and that its removal by biodegradation will be advantageous only if short residence times are used in biological treatments in wastewater treatment plants.

Key words: acenaphthene; refinery wastewaters; treatment; characterization of microbial communities; anaerobic biodegradation

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Introduction

Petroleum plays an important role in the world economy as a chemical and energy supply. Since the demand for petroleum is always increasing, the production and discharge of large volumes of oily wastewater is also growing. Therefore, refineries face the increasing environmental problem of having to treat large volumes of oily wastewater composed of hazardous compounds. In this section an overview of the recent developments concerning petrochemical wastewater problematic and treatment is presented.

1. Composition of refinery wastewaters

The composition of refinery wastewaters varies according with the origin of the petroleum as well as the processes taking place during the refining process (Dibble and Bartha, 1979). Generally, these wastewaters are complex mixtures of organic and inorganic acids, suspended particles, heavy metals, phenols, oils and greases, as well as aliphatic and aromatic hydrocarbons (Igunnu and Chen, 2013).

1.1 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are between the most dangerous and problematic pollutants, according with the Environmental Protection Agency of United States (USEPA). These compounds have many aromatic rings, composed of carbon and hydrogen atoms. The stability of aromatic PAHs with less rings, namely three and four, is lower than for PAHs with five or more aromatic rings. Additionally, the latter are less soluble in water than PAHs with three and four rings and are also more hydrophobic, and consequently have a higher tendency for adsorption on particles and sediments, whereas low molecular weight PAHs are easily dispersed in groundwater and surface water (Tsai, Kumar and Lin, 2009), leading not only to an environmental problem, but also having an impact on human health (Meckenstock, Safinowski and Griebler, 2004). The treatment of low molecular weight PAHs is therefore of high importance.

PAHs are well known for their toxicity, namely carcinogenic and mutagenic properties, and are therefore listed as priority pollutants by the US Environmental Protection Agency (United States Environmental Protection Agency 2008, Vasilieva 2011, Uad 2010).

1.2 Acenaphthene

The low-molecular weight PAH acenaphthene (Figure 1 and Table 1) is included in the list of priority substances of EPA.

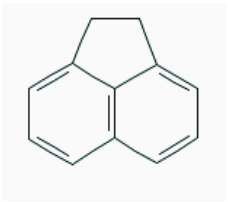


Figure 1 - Molecular structure of acenaphthene (PubChem, October 2nd 2015).

In Table 1 can be observed the properties of acenaphthene, as well as the Chemical Abstract Service Number (CAS), log K_{oc} (logarithm of soil organic carbon-water partitioning coefficient) and log K_{ow} (logarithm of octanol-water partition coefficient).

Table 1 – Properties of Acenaphthene (ChemSpider, October 18th 2015).

CAS Number	83-32-9
Molecular Formula	$C_{12}H_{10}$
Molecular Weight (g/mol)	154.21
log K_{oc}	3.79
log K_{ow}	3.92
Water solubility at 25°C (mg/L)	2.53

One of the most usual ways of acenaphthene exposure is by breathing contaminated air. Acenaphthene can irritate the skin and mucous membranes, as well as the eyes. In a 32 days study where rats were daily fed with 2 g of acenaphthene it was shown that this low quantity led to changes in rat blood, as well as in the liver, kidney and lungs. The skin also can be exposed when it contacts with contaminated soil or products that contain acenaphthene and others PAHs (EPA, September 23th 2014).

The ingestion of food or drinking water contaminated with acenaphthene is another way of be exposed, and can cause acute vomity if swallowed in big quantities (EPA, September 23th 2014).

In aerobic soil and surface waters, acenaphthene biodegradation half-life time ranges from 10 to 60 days and from 1 to 25 days, respectively. However, under anaerobic conditons or at high concentrations, acenaphthene may be persistent, since it is toxic to microorganisms. With sunlight, acenaphthene is expected to disappear by photolysis. In soils, acenaphthene can adsorb to sediments and suspended particles, as well as disappear by volatilization, which the half-lifes from a model river and a model pond are about 11 h and 39 days, respectively (ToxNet, October 12th 2015).

Even though the PAH acenaphthene is listed as a priority substance and has been found in refinery wastewaters at concentrations from less than 0.05 µg/L to 606 µg/L (Benyahia *et al*., 2006, Philemon and Benoît, 2013), there are not many studies reporting its removal. The European directive 91/271/EEC applies to the collection, treatment and discharge of urban wastewaters as well as the treatment and discharge of wastewaters from industries. But in 2000, the European Water Framework Directive (2000/60/EC) set a “zero-discharge” policy in order to control aquatic pollution. Since then, oil and gas companies have been working towards the implementation of the “zero discharge” of contaminants present in petrochemical wastewaters (Igunnu and Chen, 2013). The continuous increase of oil demand combined with more restrictive discharge regulations makes necessary the development of effective treatment technologies.

2. Treatment of petrochemical wastewaters

There are many physico-chemical processes to treat oily wastewater, namely flotation, coagulation, flocculation, sedimentation, and sorption with activated carbon. However, all of these processes are unsuitable to deal with the removal of the most toxic and effective pollutants (Dibble and Bartha, 1979). For this reason, biodegradation of many pollutants has been investigated, since it is effective and a less expensive process to remove organic contaminants from oily environments (Dibble and Bartha, 1979).

3. Biodegradation of PAHs

Biodegradation is a metabolic capacity that microorganisms have to transform or use organic pollutants as carbon source (Zhong *et al*., 2011). Petroleum hydrocarbons are the carbon and energy source of many bacteria and fungi, with a wide phylogenetic distribution, that are able to adapt to these types of compounds (Chaerun *et al*., 2004). This process involves the breakdown of organic molecules through transformation into less complex

metabolites and/or mineralization into inorganic minerals, water, carbon dioxide (aerobic) or methane (anaerobic) (Haritash and Kaushik, 2009).

Aerobic degradation implies the involvement of oxygen in the metabolic pathway. Most of the degradation pathways of aliphatic and aromatic hydrocarbons have monooxygenases catalyzing oxidation reactions, but there are also dioxygenases, enzymes that add one or two oxygen atoms to the hydrocarbons. Relatively to aromatics, the degradation ratio is related with the number of rings of the hydrocarbon. The first degradation step for many aromatic compounds is the addition of a diol group, which is cleaved afterwards, originating a carboxylic acid group that in the case of bacteria is a cis-cis diol. Polyaromatic hydrocarbons are degraded by bacteria mainly by the action of oxygenases and dehydrogenases (van der Heul, 2009).

Many of the metabolic pathways already described for PAHs biodegradation are observed under aerobic conditions, whereas anaerobic metabolic pathways are less well characterized (Tsai *et al*., 2009). In anaerobiosis, alkanes and others aromatic hydrocarbons are degraded by the bacterial synthesis pathway of fatty acids. The pathway begins with the addition of a carbon fragment to the main carbon chain, leading to a radical mechanism for further reaction that reacts with fumarate, forming a substituted succinate that can latter react through pathways very similar to the fatty acid degradation. The mechanism of the reaction is still unknown (van der Heul, 2009).

Many studies have reported that some hydrocarbons can be anaerobically oxidized when nitrate, sulfate or iron reduction, methane synthesis or photosynthesis are coupled to hydrocarbon oxidation. Many aromatic hydrocarbons, including acenaphthene, are known to be degraded under anaerobic conditions (Harayama *et al*., 1999). For toluene, all the known pathways include benzoyl-coenzyme A (CoA) synthesis. In the case of *Thauera* sp. strain T1, toluene oxidation starts by benzylsuccinate production from toluene and fumarate. After benzoyl-CoA synthesis, it is reduced by benzoyl-CoA reductase to cyclohex-1,5-diene-1-carboxyl-CoA. The subsequent steps are controversial. On another side, in *Rhodopseudomonas palustris*, cyclohex-1,5-diene-1-carboxyl-CoA is reduced to cyclohex-1-ene-1-carboxyl-CoA, while in *Thauera aromatica*, it is hydrated to 6-hydroxycyclohex-1-ene-1-carboxyl-CoA (Harayama *et al*., 1999).

Microbial biodegradation pathways are completely different under aerobic and anaerobic conditions. Berry, Francis and Bollag (1987) suggested that when microorganisms have an organic carbon source in the quantity that they need, their diversity only depends on how available its electron acceptors are, such as nitrate, sulphate, and carbon dioxide. Under anaerobic conditions, redox potential (Eh) becomes one of the main factors affecting the metabolic diversity of populations in environments like soils, sediments and aquifers. Hambrick, Delaune and Patrick (1980) demonstrated that the biodegradation rates of two petroleum hydrocarbons, namely octadecane and naphthalene, were affected by the redox potential. In this study, around 22.6% of naphthalene was degraded at Eh = 130 mV, while at an Eh of -220 mV the degradation ratio was around 0.62% at the 35-day assay.

Microbial reduction of NO_3^- to NO, N_2O and finally N_2 is called denitrification, where nitrate is the final electron acceptor. Denitrification is common in the oxidation of organic compounds, and usually the final product of the reaction is nitrogen. Denitrifying microorganisms have been reported in some anoxic environments, such as soils and oceans. Denitrification can occur when oxigen dissolved is present at concentrations lower than 10 μM and can take part of biodegradation pathways of organic pollutants (Karthikeyan and Bhandari, 2001).

Mihelcic and Luthy (1988) reported for the first time naphthalene biodegradation under denitrifying conditions. The authors analised biodegradation of acenaphthene and naphthalene, at soil-water ratio of 1:25 and 1:50. With nitrate in excess, both PAHs were biodegraded to less than 0.01 mg/L in less than nine weeks, where an acclimation time of 12 to 36 days in soils not previously contaminated with PAHs was observed. This was not observed in soils with a historical of PAHs contamination. These results were also an indication that PAH biodegradation using nitrate as electron acceptor depends on the ratio of PAH relatively to the other carbon

sources. Al-Bashir *et al*. (1990) studied naphthalene biodegradation in a soil-slurry system under denitrifying conditions, with an initial concentration of 50 mg/L, which around 90% was biodegraded within 50 days, and with the highest biodegradation ratio hitting 1.3 mg/L per day. In a recent study, pure cultures of denitrifying bacteria – NAP-3-1 and NAP-4 – were also identified and isolated with the ability to anaerobically biodegrade PAHs, namely phenanthrene, naphthalene and biphenyl, where naphthalene relevant biodegradation occurred only in the presence of nitrate. The isolate NAP-3-1 was found to be a denitrifier bacteria, because significant concentrations of nitrogen were produced, while NAP-4 produced nitrite also at significant concentrations. This was the first study reporting nitrate-dependent anaerobic naphthalene biodegradation by pure cultures (Rockne *et al*., 2000).

Sulfate reduction is another pathway of anaerobic biodegradation, also named sulfidogenesis, where microorganisms consume low molecular weight organic acids, alcohols, and H_2 as electron donors (Karthikeyan and Bandhari, 2001). Sulfate reducing microorganisms are strictly anaerobes and they are present not only in freshwater and marine sediments but also in soils, besides they are more frequent in the marine environment, because there is much more sulfate than oxygen, nitrate and ferric iron in the sediments, being sulfidogenesis the predominant terminal electron-accepting progress (Karthikeyan and Bandhari, 2001). Some studies of PAHs biodegradation under denitrifying conditions reported that sulfate reduction does not contribute to PAHs degradation, which was not reported in sulfate-reducing conditions until 1996, by Coates *et al*., where not only was reported naphthalene and phenanthrene degradation to CO_2 under strict anaerobic conditions, which sample was sediments from San Diego Bay, California, but also that sulfate reduction was essential for PAH degradation in this sample. The same authors also reported the anaerobic biodegradation of methylnaphthalene, fluorene and fluoranthene, in the same sediments (Coates *et al*., 1997), where naphthalene biodegradation was sulfate dependent. In sediments from a less contaminated place in San Diego Bay, where PAHs were not readily degraded, the authors inoculated the sediments with the cultures of the heaviest contaminated place, and observed PAHs biodegradation. These results suggest that sulfate reduction can be used for the treatment of marine sediments contaminated with PAHs, where microorganisms that use sulfate as electron acceptor could be used.

It has been suggested that carboxylation is the initial reaction in the anaerobic biodegradation of PAHs under sulfate-reducing conditions. Carboxylation leads to the synthesis of benzoate-like analogs, the better substrate for activation by coenzyme A binding, which is followed by ring reduction. PAH carboxylation consolidate the idea that reductive hydrogenation happens only after the destabilization of the aromatic ring, or its activation by carboxilation. If carboxylated PAHs are metabolized by sequential ring reduction and ring fission, as it occurs with benzoate under anaerobic conditions, is still unknown (Karthikeyan and Bhandari, 2001).

Zhang and Young (1997) identified the main metabolites of degradation of naphthalene and phenanthrene, which was 2-naphthoic acid and phenanthrene-carboxylic acid, in a sulfate-reducing enrichment culture, which also was verified by Meckenstock, Safinowski and Griebler (2004), showing that 2-naphthoic acid is an intermediate of naphthalene degradation pathway. More metabolites of the pathway were identified such as 1,2,3,4-tetrahydro-2-naphthoic acid, 5,6,7,8-tetrahydro-2-naphthoic acid, hexahydro-2-naphthoic acid, octahydro-2-naphthoic acid and decahydro-2-naphthoic acid. The reduction pathway is thought to proceed with 5,6,7,8-tetrahydro-2-naphthoic acid, in agreement with a naphthalene-degrading culture analysis, where this compound was found as the major metabolite. Another study of naphthalene degradation, with a marine and sulfate-reducing culture, also showed that this was the metabolite of 2-naphthoic acid degradation. This discover indicated that the ring cleavage should begin in the beta-position of the carboxyl group of 5,6,7,8-tetrahydro-2-naphthoic acid, which results in the production of a compound like a cyclohexane. This is in agreement with previous experiences, where two degradation products with a cyclohexane ring and two carbonic acid side chains, respectively, were identified (Meckenstock, Safinowski and Griebler, 2004).

Biodegradation of fluorene and phenanthrene was reported by sulfate-reducing bacteria, that growed for nearly 4 years with lactate as carbon source in the enrichment phase. Biodegradation ratios were measured in mixture and with the single PAHs during 9 days and from day 10 to 21 days. During the process, was observed a decrease of the initial sulphate concentration (8.8-17.8%), what means that PAHs were biotransformed simultaneously with sulfate reduction (Tsai, Kumar and Lin, 2009). Methanogenesis consists in the conversion of low molecular weight organic acids to methane, and it is a strictly anaerobic pathway. The availability of electron donors can lead to competition between sulfidogenic and methanogenic microorganisms, since when it is low, sulfate-reducing bacteria are predominant at sulphate concentrations lower than 1 mg/L, whereas when it is high methanogens are predominant (Karthikeyan and Bhandari, 2001). The degradation of unsubstituted PAH in methanogenesis it is not frequent, since only compounds with polar substituents such as naphthol can be metabolized (Meckenstock, Safinowski and Griebler, 2004).

The first step of the anaerobic biodegradation pathway of toluene by *Azoarcus* sp. and *Thauera* sp., which are denitrifying bacteria, is the addition of fumarate to the methyl group of toluene. This reaction is mediated by benzylsuccinate synthase, that removes a hydrogen atom from the methyl group of toluene and add it to the C-2 atom of fumarate (Meckenstock, Safinowski and Griebler, 2004).

Yuan and Chang (2007) reported the anaerobic biodegradation of a mix of five PAHs, namely acenaphthene, fluorene, phenanthrene, anthracene and pyrene. The authors verified that the biodegradation was higher when the PAHs were mixed than when individually assessed. This can be due the higher availability of PAHs, since there are many carbon sources available. The effectiveness of the degradation in the mixture was the following by decreasing order: acenaphthene (0.2 µg/g/day), fluorene (0.01 µg/g/day), phenanthrene (0.07 µg/g/day), anthracene (0.03 µg/g/day) and pyrene (0.01 µg/g/day). The authors also tested the effectiveness of the PAHs mixture biodegradation using three different carbon sources, namely lactate, acetate and pyruvate, under methanogenic, sulfate-reducing and nitrate-reducing conditions. Comparatively to the inoculum, for all the PAHs and all carbon sources, the highest biodegradation enhancing was observed under sulfate-reducing conditions, and by last under methanogenic conditions, since the biodegradation was inhibited under nitrate-reducing conditions. The order of carbon sources for the biodegradation of acenaphthene under sulfate-reducing conditions was the following: acetate (0.54 µg/g/day), lactate (0.49 µg/g/day) and pyruvate (0.41 µg/g/day). The authors found that all these carbon sources promote the growth of methanogenic bacteria, leading to degradation enhancement in methanogenic conditions. On the other hand, acidogenic metabolism of lactate or pyruvate produces hydrogen or hydrogen carbonate, which stimulates methanogen production (Yuan and Chang, 2007). Relatively to sulfate-reducing conditions, bacteria that use lactate produce pyruvate plus two electrons as final products, while pyruvate action promotes acetate plus two electrons as final products, both of which will improve sulfate-reducing degradation (Yuan and Chang 2007), while nor lactate, acetate or pyruvate are carbon sources in nitrate-reducing conditions, inhibiting PAHs degradation (Yuan and Chang, 2007).

3.1 PAH degrading microorganisms

Bacteria from different phylogenetic groups have been detected in petrochemical wastewaters (Haritash and Kaushik, 2009, Silva *et al*., 2013,) and several authors reported their ability to remove hydrocarbons (Lopes-Oliveira *et al*., 2012, Mazzeo *et al*., 2010, Meckenstock, Safinowski and Griebler, 2004). The most efficient bacteria present in petroleum that have been reported to remove aliphatic and aromatic hydrocarbons, under aerobic conditions, are: *Rhodococcus erythropolis*, *Micrococcus luteus*, *Pseudomonas putida*, *Pseudomonas fluorescens,* and *Mycobacterium frederikbergense* (Steliga, Jakubowicz and Kapusta, 2012). These bacteria are members of phylum *Proteobacteria*, namely of the class *Gammaproteobacteria*, at the case of *Pseudomonas*

spp., whereas *Rhodococcus erythropolis*, *Micrococcus luteus* and *Mycobacterium frederikbergense* belong to the phylum *Actinobacteria*. Also *Archaea* were already described in beach sediments and spilled oil (Roling *et al*., 2004). In another study under aerobic conditions of crude-oil biodegradationin soils, *Actinobacteria*, namely *Rhodococcus erythropolis* and *Rhodococcus coprophilus*, knowns to degrade hydrocarbons, were found in the soil with highest ratio of n-alkanes degradation (81% by the 10^{th} essay day), coincident with the shortest latency phase of bacteria (Hamamura *et al*., 2006).

Under aerobic conditions, acenaphthene degradation was reported by a strain of *Ochrobactrum* sp., of the class *Alphaproteobacteria*, three strains of *Brevibacillus parabrevis*, of *Bacilli* class (Bao *et al*., 2012), and also *Beijerinckia* sp. and *Pseudomonas* spp., belonging to *Alphaproteobacteria* and *Gammaproteobacteria* respectively (Cerniglia, 1992). Some genes involved in acenaphthene degradation have been reported in the strictly aerobic *Sphingomonas* sp., an *Alphaproteobacteria* known by its existance in soils, water and sediments and for having the ability of using acenaphthene as only carbon and energy source (Kouzuma *et al*., 2006). Acenaphthene degradation, as well as acenaphthylene, was also reported in *Beijerinckia* sp., which aerobic metabolic pathway was reported by Schocken and Gibson (1984). Acenaphthene oxidation is also reported in the following *Proteobacteria*: *Beijerinckia* sp., of the class *Alphaproteobacteria*, the six *Gammaproteobacteria*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Pseudomonas* sp., *Cycloclasticus* sp., *Neptunomonas naphthovorans* and *Burkholderia cepacia*, and also *Alcaligenes eutrophus* and *Alcaligenes paradoxus*, both of them members of the class *Betaproteobacteria* (Juhasz and Naidu, 2000), and *Acinetobacter* sp., a *Gammaproteobacteria* in which is characterized the aerobic metabolic pathway (Ghosal *et al*., 2013).

McNally, Mihelcic and Lueking (1998) determined that *Pseudomonas fluorescens* W2, *Pseudomonas putida* KBM1 and *Pseudomonas stutzeri* SAG-R were able to degrade three and four-rings PAHs under denitrifying conditions to undetectable levels. The authors tested *Pseudomonas fluorescens* and *Pseudomonas putida* for biodegradation of a mixture of naphthalene (3 mg/L), acenaphthene (3 mg/L), anthracene (0.045 mg/L) and pyrene (0.13 mg/L). Both bacteria degraded naphthalene to levels below detection in 6 to 8 h and acenaphthene was degraded by both *Pseudomonas fluorescens* and *Pseudomonas putida* after 80 h. Biodegradation under denitrifying conditions was demonstrated by the steady concentration of nitrate after 58 h, by the time that all the PAHs have been degraded to undetectable levels.

It has been observed that PAHs degradation, namely naphthalene and phenanthrene, as well as others hydrocarbons, is possible under anaerobic conditions by methanogenic bacteria affiliated with *Thermotogales*, *Synergistales* and *Defferibacterales*, members of the phyla *Thermotogae*, *Synergistetes* and *Deferribacteres* respectively. The bacterium *Anaerobaculum hydrogeniformans*, also member of *Synergistetes* phylum (Gieg *et al*., 2010) and also *Sphingomonas* sp., *Rhodobacter* sp. and *Roseobacter* sp. are reported as polyaromatic hydrocarbon degraders (Meslé, Dromart and Oger, 2013), curiously all of them *Proteobacteria*, namely *Alphaproteobacteria*.

Rhodococcus sp. (Walter *et al*., 1991) and *Mycobacterium* sp. (Schneider *et al*., 1996) have been described as benzo[a]pyrene degraders (Trzesicka-Mlynarz and Ward, 1995). *Clostridium* sp. also was reported as acenaphthene degrader in a mix of five PAHs (acenaphthene, fluorene, phenanthrene, anthracene and pyrene), since the most effective strain degraded completely acenaphthene and fluorene of the mixture (concentration of 1 µg/g either alone or in mixture), in a period of 72 days (Yuan and Chang, 2007) under anaerobic conditions.

3.2 Factors affecting biodegradation

Bioremediation, one of the best approaches to clean petroleum contaminated environments, attempts to accelerate the naturally occurring biodegradation of contaminants through the optimization of limiting conditions (Margesin and Schinner, 2001).

Bioremediation of a pollutant and its rate depends on the number and type of the microorganisms, nature and chemical structure of the contaminants, as well as environmental conditions (Haritash and Kaushik, 2009). Therefore, several factors need to be accounted to achieve the successful removal of a micropollutant: pH, temperature, oxygen, microbial population, degree of acclimation, accessibility of nutrients, chemical structure of the compound, cellular transport properties, and chemical partitioning in growth medium (Haritash and Kaushik, 2009).

Temperature is an important factor due to the enzymes involved in the degradation pathway that have an optimum activity temperature and consequently this factor influence the metabolic turnover (van der Heul, 2009). The low temperatures lead to an increase of oil viscosity, a reduction in the volatilization of toxic short-chain alkanes and an increase in water solubility of these compounds, resulting in a smaller degradation ratio at low temperatures. Normally, low temperatures lead to a decrease of the enzymatic activity, whereas higher temperatures allow microorganisms to reach the maximum rate of hydrocarbon metabolism, which commonly occurs between 30°C and 40°C, since above this temperature range hydrocarbons have a higher toxicity for cell membranes. In fact, the low winter temperatures were considered the limiting factor in the biodegradation of polyaromatic hydrocarbons in estuarine sediments (Shiaris, 1989) and of many hydrocarbons in freshwater lakes (Leahy and Colwell,1990).

Oxygen is the determinant factor for aerobic or anaerobic reaction to take place (van der Heul, 2009). Furthermore, oxygen concentration was already identified as the limiting factor for the biodegradation ratio of petroleum in soil and the biodegradation of gasoline in groundwater. Relatively to marine and freshwater environments, in upper levels of the water column, oxygen concentration is generally high. Moreover, sediments are normally anoxic, except for the thin layer at the sediment surface. The oxygen available in soils depends of its microbial consumptiom, the type of soil, if the soil is waterlogged or not, and the presence of substrates that lead to oxygen depletion. Recent studies showed that microbial consortia from soil and sludge metabolize many aromatic hydrocarbons in anaerobiosis, including benzene, toluene, acenaphthene and naphthalene. Furthermore, the hydrocarbons removal can be relevant, since at least 50% of benzene and toluene were biodegraded in 60 days under methanogenic conditions, and naphthalene and acenaphthene reached non-detectable levels in 45 and 40 days, respectively, under denitrifying conditions (Leahy and Colwell,1990).

Was done a study where seven strains of facultative anaerobic nitrate reducing bacteria, isolated from polluted soils, were assessed in terms of their potential to degrade hydrocarbons, which five of the seven strains were determined as *Pseudomonas* sp. and the others two as *Brevundimonas* sp. The study showed that aerobic degradation is much faster than anaerobic degradation. Furthermore, only biodegradation of naphthalene and fluorene has been observed in anaerobiosis. These were a ten day aerobic and a fifty day anaerobic experiments. Bacteria growing in the presence of oxygen were able to degrade 90-95% of the alkanes and a statistically relevant degradation of PAHs was not observed. At the 50^{th} day under anaerobic conditions, 20-25% of the alkanes were degraded and biodegradation of naphthalenes was between 13% and 18%, while it was 23% for fluorene. It was also shown that the degradation of alkanes of intermediate length was higher than the degradation of shorter and longer alkanes (Grishchenkov *et al*., 2000).

Nutrients are another factor with importance in anaerobic biodegradation. The release of hydrocarbons into aquatic environments with low concentrations of inorganic compounds leads to very high ratios of carbon/nitrogen

or carbon/phosphorous, which negatively impact microbial growth. It is known that the concentration of nitrogen and phosphorus available for microorganisms limits the biodegradation of hydrocarbons in water environments as estuaries, sea, freshwater lakes, Arctic ponds, and groundwater. It was observed that the addition of nitrogen and phosphorus enhanced the biodegradation of crude oil and its hydrocarbons in seawater, Arctic ponds and lakes. On the other hand, besides inorganic salts of nitrogen and phosphorus are effective in enclosed systems, they tend to wash out in experiments *ex situ* (Leahy and Colwell,1990).

Salinity also has influence in the biodegradation efficiency and microorganisms present. Normally, higher salinity is associated with higher biodegradation efficiency, according with Shiaris (1989), that studied the biodegradation of phenanthrene, naphthalene and benzo[a]pyrene in sediments of an estuary. However, other studies reported the opposite correlation, namely in hypersalines salt evaporation ponds, in which the metabolic rates for hydrocarbons decreased with the increases in salinity, what was determined as a consequence of the reduction in microbial metabolism (Ward and Brock, 1979).

It is thought that pressure is an important factor in the biodegradation of hydrocarbons when we are talking about deep-sea environment. There are few studies about the impact of pressure in the biodegradation efficiency, since only the studies of Schwarz, Walker and Colwell (1974) are known. The authors analysed the degradation of hexadecane by a mixed bacteria culture of deep-sea sediment, at a pressure of 1 atm, and 495 or 500 atm. At 4°C, there was a biodegradation of 94% of hexadecane under higher pressures in 40 weeks, where at 1 atm the same efficiency was reached in 8 weeks, revealing that there is a negative correlation between pressure and hydrocarbon biodegradation (Leahy and Colwell,1990).

In water, the pH is relatively stable. Most heterotrophic microorganisms have an optimal growth at neutral pH or near it, so extreme pH values are expectable to negatively impact the effectiveness of hydrocarbons degradation. Was tested the biodegradation of gasoline in an acidic soil (pH 4.5), and observed a very low biodegradation ratio. However, when the pH was adjusted to neutral values (7.4), the biodegradation ratio was the double of the observed in the acidic sample, what unexpectly was not observed with pH values of 8.5 (Leahy and Colwell,1990). These results are in agreement with another study of mineralization of oily sludge in soils, where the optimal pH was of 7.8, in a range of 5.0 to 7.8 (Dibble and Bartha, 1979).

A previous exposure of the microbial communities to hydrocarbons is one of the most important factors for the velocity of biodegradation in case of exposition to contaminants, which contributes for an enhancement in the hydrocarbon biodegradation ability of a community, also called of adaptation. This phenomenon can occur by three different but related ways: induction and/or repression of the enzymes responsible by biodegradation; genetic changes that lead to the acquisition of new metabolic pathways; selective enrichments of microorganisms that are able to use the compound. Many studies have reported not only that after exposure to crude pollutants hydrocarbon biodegraders are in bigger number, but also that its abundance is related with the degree of contaminantion of its ecosystem. Sometimes, adaptation reflects a higher abundance of certain biodegraders genera, but by another hand, it has already been observed that heterotrophic populations have a tendency to be diverse and maintain or increase their diversity after a contamination (Leahy and Colwell,1990).

4. Objectives and thesis outline

The main objective of the work developed and presented in this thesis was to identify a consortium of bacteria from a refinery wastewater that is able to remove the PAH acenaphthene under anaerobic conditions.

This thesis is divided in three parts: Chapter 1 – Introduction - consists of a revision of the state-of-the-art focusing on the composition of refinery wastewaters, occurrence and toxicity of PAHs (particularly acenaphthene), as well as biological treatment (efficiency and degrading bacteria); Chapter 2 – Materials and Methods –describes

all the experimental details of the work developed; Chapter 3 – Results and Discussion – presents and discusses the results obtained at this work.

Chapter 3 is divided in three main sections. The aim of the first section was to address the best enrichment strategy in terms of inoculation strategy and carbon source as well as to identify the most suitable bacteria consortium for following experiments. Microbial communities profiles of the raw wastewater and enriched cultures were attained by next-generation sequencing and compared. In the second section, the stability of acenaphthene and respective toxicity towards microbial growth was evaluated with the objective of establishing the acenaphthene concentration to be applied in following biodegradation experiments. In the third section, biodegradation experiments were conducted in the presence and absence of an extra carbon source (lactate) to evaluate the degrading potential of the microbial consortium under both conditions and assess the respective microbial profiles.

Materials and Methods

1. Chemicals

The following reagents with the highest purity available (> 98%) were used to prepare culture media: D-(+)-glucose (Sigma Chemical CO., USA), ascorbic acid (Sigma Chemical CO., USA), ammonium chloride (Panreac Quimica SA, Spain), sodium sulphate anhydrous (Panreac Quimica SA, Spain), potassium di-hydrogen phosphate (Panreac, Spain), iron II sulphate heptahydrate (Panreac, Spain) calcium chloride-dihydrate (Merck, Germany), trisodium citrate dihydrate (Merck, Germany), sodium lactate solution 50% (Merck, Germany), magnesium sulfate heptahydrate (Fluka, Japan), yeast extract (Biokar Diagnostics), sodium thioglycolate (ACROS ORGANICS, USA) and sodium nitrate (Chem-Lab NV, Belgium). Resazurin (Sigma-Aldrich CO., USA) was added to the medium as anaerobiosis indicator. Sodium hydroxide 1 M was used to carry out pH adjustments.

High performance liquid chromatography (HPLC) grade acetonitrile (Fisher, UK) was used for the chromatographic analysis of acenaphthene, as well as for the preparation of stock and intermediate solutions. Milli-Q water used in the chromatographic analysis and in the preparation of culture media was produced by a Milli-Q water system (Whatman, Nylon, 0.2 µm, 47 mm, Germany).

2. Refinery wastewater

The wastewater used in the work described in this thesis was collected from the GALP refinery, located in Sines, after the flotation treatment of the global effluent, which results from the combination of all effluents from the oil refining process.

3. Enrichment of microbial community

3.1 Preparation of culture medium

The composition of the culture medium used for the enrichment of the microbial community under anaerobic conditions was set based on previous experience in the laboratory (Table 2).

Table 2 - Composition of the culture medium used for microbial enrichment.

Compound	Concentration
Potassium Dihydrogen Phosphate (KH_2PO_4)	0.5 g/L
Ammonium Chloride (NH_4Cl)	1 g/L
Calcium Chloride Dihydrate ($CaCl_2.2H_2O$)	0.06 g/L
Magnesium Sulphate Heptahydrate ($MgSO_4.7H_2O$)	0.06 g/L
Iron (III) Sulfate Heptahydrate ($Fe_2SO_4.7\ H_2O$)	0.0071 g/L
Yeast extract	0.2g/L
Trisodium Citrate ($Na_3C_6H_5O_7$)	0.3 g/L
Ascorbic Acid ($C_6H_8O_6$)	0.1 g/L
Sodium thioglycolate ($C_2H_3NaO_2S$)	0.1 g/L
Sodium Sulfate (Na_2SO_4)	20 mM
Sodium Nitrate ($NaNO_3$)	20 mM
Glucose ($C_6H_{12}O_6$) or Lactate ($C_3H_6O_3$)	80 mg/L
Resazurine ($C_{12}H_7NO_4$)	107.5 mg/L

Resazurine was used as anaerobiosis indicator since it is pink in the presence of oxygen and colorless in the absence of oxygen. Trisodium citrate, ascorbic acid, and sodium thioglycolate were used as reducing agents, while sulphate and nitrate were used as potential electron acceptors of anaerobic metabolism.

All the components detailed above were weighted in an analytical scale (Sartorius, BP2100S, USA) and added to a gobelet where the adequate amount of Milli-Q water was added to achieve the required concentrations. Magnetic stirring was used to ensure solubilization of all components, followed by pH adjustment at 7.0-7.2 with a 1 M sodium hydroxide solution using a potenciometer (Crison instruments, S.A., micropH 2002, Spain). Then, the medium was distributed in 1 L anaerobiosis flasks, degassed under a nitrogen stream, and sealed with common flasks caps containing a rubber in the central part. The culture medium was considered anaerobic when it was colorless. The flasks were then sterilized at 120°C during 20 min. Lactate and glucose 1 M stock solutions were then filtered using 0.45 µm sterile filters (Milipore, Type HA, 47 MM, USA), and added to the corresponding flasks (see section 3.2) under anaerobic and sterile conditions to achieve the required concentrations.

3.2 Innoculation

Two different approaches were followed for the enrichment of the microbial community present in the refinery wastewater under anaerobic conditions. In the first approach, two reactors containing 250 mL of sterile culture medium each were directly inoculated with 250 mL of refinery wastewater under anaerobic conditions using a syringe and a needle. These reactors only differed in terms of the carbon source: glucose (reactor A) or lactate (reactor B). Since these reactors are composed of 50% culture medium and 50% wastewater, the concentration of the components in the culture medium was doubled relatively to what is detailed in Table 2 in order to achieve those final concentrations after inoculation.

Figure 2 – Reactors of the enrichment assay.

In the other approach, 1.5 L of wastewater were centrifuged (Labnet International, Inc, Spectrafuge 24D, USA) at 4000 rpm during 10 min to concentrate the biomass. The pellet was ressuspended in 100 mL of sterile culture medium and then, equally distributed in two reactors containing 450 mL of culture medium each under anaerobic conditions using a syringe and a needle. As in the first approach, these two reactors also differed only in terms of the carbon source: glucose (reactor C) or lactate (reactor D). In this approach, the inoculum volume corresponds to 10% of the total reactor volume.

All reactors were spiked with the appropriate volume of acenaphthene stock solution to achieve a concentration of 100 µg/L. Then, all reactors were incubated in the dark (to avoid photodegradation of acenaphthene). Whenever the concentration of acenaphthene was found low, another spike was carried out to ensure selective pressure. The assays were performed at room temperature (21.4 ± 0.86) during 17 days (Table 2). Optical density at 600 nm was measured daily using a spectrophotometer (Biochrom Ltd., Ultrospec 2100 pro, UK) to follow microbial growth. Samples of 1 mL were also collected and filtered using 0.45 µm sterile filters (Milipore, Type HA, 47 MM, USA) for HPLC analysis to evaluate the variation of the concentration of acenaphthene in the reactors.

Samples were refrigerated at 4°C until analysis. In the 17th day of incubation, 50 mL (10%) of the cultures in reactors A to D were individually inoculated in 450 mL of fresh sterile culture media with the same composition described in Table 2. A third inoculation was done, using the cultures attained in the end of the second inoculation, to remove particles and organics present in the petrochemical wastewater.

A stock culture was prepared for each reactor. For that, several vials were prepared containing 100 µL of the respective microbial culture collected in the exponential growth phase and 900 µL of glycerol. The vials were immediately immersed in liquid nitrogen, removed with a tweezer after freezing, and stored at -80ºC for subsequent experiments.

3.3 Characterization of microbial communities

Next-generation sequencing (NGS) of 16S ribosomal RNA was conducted by Biopremier (Portugal), using an Ion Torrent equipment (Thermo Fisher Scientific, USA), to assess the dynamics of the microbial communities during the enrichment. For that, samples of the petrochemical wastewater as well from reactors A to D were collected in the end of enrichment.

4. Resistance assays

Resistance assays were carried out to assess the resistance of the enriched microbial community from reactor D towards acenaphthene as well as the stability of this PAH. In this view, these tests gave insights on the acenaphthene concentration to be used in subsequent biodegradation assays.

4.1 Preparation of the inoculum

The inoculum used in this assay was prepared as follows: A stock culture (1 mL) was thawed and immediately inoculated in a 10 mL tube containing culture medium with lactate (prepared as described in section 3.1), as well as 100 µg/L of acenaphthene under anaerobic and sterile conditions. The culture was allowed to grow during 24 h (time necessary to achieve maximum growth) in the dark at room temperature. Then, 3 mL of this pre-inoculum were added to a 30 mL reactor containing the same culture medium and acenaphthene. Another incubation period was carried out during 24 h under the same conditions.

4.2 Assessment of acenaphthene toxicity and stability

Nine reactors containing 30 mL of the culture medium with the same composition used for inoculum growth were assessed (Table 3).

Table 3 - Reactors evaluated during resistance tests.

Reactors	Designation	Aim
A	Inoculum + 100 µg/L acenaphthene	Test acenaphthene toxicity
B	100 µg/L acenaphthene	Test acenaphthene stability (abiotic control)
C	Inoculum + 500 µg/L acenaphthene	Test acenaphthene toxicity
D	500 µg/L acenaphthene	Test acenaphthene stability (abiotic control)
E	Inoculum + 1000 µg/L acenaphthene	Test acenaphthene toxicity
F	1000 µg/L acenaphthene	Test acenaphthene stability (abiotic control)
G	Inoculum + 1500 µg/L acenaphthene	Test acenaphthene toxicity
H	1500 µg/L acenaphthene	Test acenaphthene stability (abiotic control)
I	Inoculum	Test community growth (biotic control)

All reactors, except reactor I, were spiked with the adequate volume of acenaphthene stock solution to achieve concentrations of approximately 100, 500, 1000, and 1500 µg/L.

Figure 3 – Reactors used in resistance assays.

These concentrations were set based on the occurrence concentrations detailed in the Introduction section [(from 0.05 µg/L (Benyahia *et al*., 2006) to 606 µg/L (Philemon and Benoît, 2013)], as well as the solubility of acenaphthene in water [(3.9 mg/L (ChemSpider)]. A volume of 3 mL of the inoculum prepared as detailed in section 4.1 were also added to reactors A, C, E and G to assess acenaphthene toxicity to bacteria, and to reactor I to test community growth in the absence of acenaphthene (Table 3). Reactors B, D, F and H only contained acenaphthene in order to test acenaphthene stability at the mentioned concentrations. All reactors were incubated in the dark at room temperature (21.4°C ± 0.86) during 7 days. Temperature measurements were taken two to three times a day. Optical density at 600 nm was measured daily by collecting 1 mL samples; two measurements were conducted for each sample and the average value was determined. Samples of 1 mL were also daily collected for HPLC analysis. Standard solutions with the following concentrations were prepared for HPLC analysis using culture medium with the same composition of the medium contained in the reactors: 0, 5, 10, 50, 100, 500, 1000, 1500, and 1800 µg/L.

5. Biodegradation assays

Biodegradation assays were carried out to evaluate the potential removal of acenaphthene by the microbial culture used in the resistance tests in the presence and absence of an extra source of carbon (lactate).

5.1 Culture medium

In the biodegradation assay, the culture medium was changed since ascorbic acid, trisodium citrate and sodium thioglycolate were removed. This change was based on a study taking place at the same time in the laboratory, where this culture was able to grow faster in the absence of these reducing agents. The other components of the culture medium were used at the same concentrations (Table 4).

Table 4 - Composition of the culture media used for the biodegradation assay.

Compound	Concentration
Potassium Dihydrogen Phosphate (KH_2PO_4)	0.5 g/L
Ammonium Chloride (NH_4Cl)	1 g/L
Calcium Chloride Dihydrate ($CaCl_2.2H_2O$)	0.06 g/L
Magnesium Sulphate Heptahydrate ($MgSO_4.7H_2O$)	0.06 g/L
Yeast extract	0.2g/L
Iron (III) Sulfate Heptahydrate ($Fe_2SO_4.7\ H_2O$)	0.0071 g/L
Sodium Sulfate (Na_2SO_4)	20 mM
Sodium Nitrate ($NaNO_3$)	20 mM
Lactate ($C_3H_6O_3$)	80 mg/L
Resazurine ($C_{12}H_7NO_4$)	107.5 mg/L

5.2 Preparation of inoculum

The inoculum used in the biodegradation assay was prepared as described in section 4.1. However, a 60 mL reactor was used instead of 30 mL and therefore, 6 mL of pre-inoculum were used to inoculate 60 mL reactors. Furthermore, the microbial culture was washed before its addition to the biodegradation reactors to ensure the removal of residual lactate and acenaphthene since some of the reactors did not contain lactate. For that, 18 mL of inoculum were collected at the exponential growth phase and centrifuged at 4000 rpm during 10 min. Biomass was, then, ressuspended in 3 mL of fresh sterile medium without lactate, and 1 mL of this suspension was added to certain reactors.

5.3 Assessment of acenaphthene removal

Five reactors containing 60 mL of culture medium were prepared with or without lactate (Table 5).

Table 5 - Reactors evaluated during biodegradation assay.

Reactor	Designation	Aim
A	Inoculum + 100 µg/L acenaphthene + lactate	Test biodegradation with lactate
B	100 µg/L acenaphthene + lactate	Test adsorption/Effect of lactate in acenaphthene
C	Inoculum + 100 µg/L acenaphthene	Test biodegradation with acenaphthene as only carbon source
D	100 µg/L acenaphthene	Test adsorption of acenaphthene
E	Inoculum	Analyze initial community

Reactors B and D were not inoculated to test the removal of acenaphthene in the presence and absence of lactate by other mechanisms besides biodegradation. Reactor E was used as the biotic control. This control was therefore the only reactor that was not spiked with 100 µg/L of acenaphthene. This concentration was selected for

biodegradation assays based on resistance assays. All reactors were incubated in the dark at room temperature (24°C ± 0.5) during 31 days.

Optical density at 600 nm was measured daily after collecting 1 mL sample from each reactor; two measurements were conducted for each sample and the average value was determined. Another 1 mL sample was also daily collected for HPLC analysis. Two sets of standard solutions were prepared for HPLC analysis that differed only in terms of the presence or absence of lactate and presented the following acenaphthene concentrations: 0, 5, 10, 50, 100, and 200 µg/L.

5.4 Characterization of microbial communities

NGS was carried out as described in section 3.3 to assess the dynamics of the microbial communities during biodegradation assays in the presence and absence of lactate. For that, samples were collected from the reactor where the microbial culture used as inoculum was grown (taken at time 0 of the biodegradation assay) as well as from reactors A and C in the end of the experiment.

6. Analytical Methods

Samples were analyzed by direct HPLC injection for the determination of acenaphthene concentration using a Waters system (Alliance e2695 Separations Module) equipped with a Multi Fluorescence Detector (2475, Waters Chromatography, Milford, MA, USA) and a HyperClone 5 µm PAH (250 mm × 4.6 mm) column (Phenomenex Inc., Torrance, CA, USA). The analysis of acenaphthene was carried out under isocratic conditions with a flow rate of 1.5 mL/min and mobile phase composed of 55% acetonitrile and 45% milli-Q water. The injection volume was 50 µL, the oven temperature was set at 40°C and acenaphthene was monitored by fluorescence, using emission and excitation wavelengths of 340 and 225 nm, respectively. The detection limit of the method is 5 µg/L.

For each analysis, calibration standards (0, 5, 10, 50, 100 and 200 µg/L) were prepared in the same culture medium used in the reactors, with or without lactate, depending on the reactors from where samples were taken.

Results and Discussion

1. Enrichment assay

In this assay, two different approaches were followed to attain a microbial community that is able to grow under anaerobic conditions in culture medium and that could be used in biodegradation assays. In the first approach, two reactors containing sterile culture medium were directly inoculated with refinery wastewater while in the second approach two reactors containing culture medium were inoculated with centrifuged biomass. These two different approaches were followed to assess if the microbial community originally present in the wastewater could adapt to the culture medium composition or needed nutrients from the original wastewater. For that, microbial growth rates, as well as the community profiles were assessed and compared. In both approaches, two different carbon sources were addressed – glucose and lactate - to determine the best carbon source for the microbial growth. Reactors were codified as: (A) 50% of refinery wastewater and glucose, (B) 50% of refinery wastewater and lactate, (C) 100% of culture medium and glucose, (D) 100% of culture medium and lactate.

1.1 Microbial growth

Figure 4 shows the growth curves attained at 600 nm over time in reactors A to D during the first enrichment.

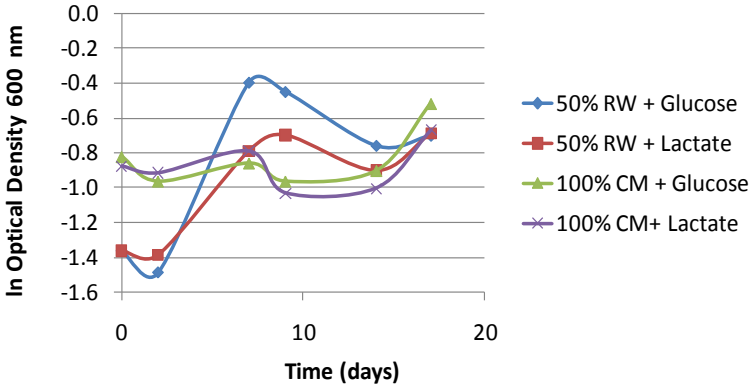


Figure 4 - Logarithm nepperian of the optical density attained at 600 nm during the microbial enrichment in reactors A to D. RW: Refinery Wastewater; CM: Culture Medium

Figure 4 shows that the microbial growth was faster in the reactors containing 50% of refinery wastewater (A, B) despite the initial lower absorbance comparatively with the other reactors (C, D) since i) the exponential phase is more pronounced (A: μ=0.06; B: μ=0.05; C: μ=0.003; D: μ=0.001), with a higher maximum absorbance value, and ii) shorter lag and stationary phases were observed. Furthermore, a stationary phase is observed between the 9th and 17th days for reactors A and B. The higher initial absorbance determined at time 0 for reactors C and D is related with the high volume of wastewater that was centrifuged and that led to a very concentrated inoculum.

These results indicate that the adaptation of the microbial community to the enrichment conditions might not have been as easy in the reactors containing 100% of culture medium (C, D) as in the other reactors (A, B). Therefore, the presence of compounds originally from the refinery wastewater is likely to have supported a faster adaptation and consequently, a more pronounced growth since the change in the composition of the medium was less drastic. Similar absorbance values were, however, determined at the 17th day for all reactors addressed.

Regarding the carbon source, the maximum microbial growth was observed with glucose in the reactor containing 50% of refinery wastewater (A). However, the decline phase is less pronounced in the reactor containing lactate (B) (Figure 4). It seems that although glucose promotes a faster growth, the stationary phase is longer in the presence of lactate. Fairly similar growths were observed for the other reactors (C, D), independently of the carbon source.

Lactate has been reported in the literature as a carbon source for some anaerobic bacteria (Hippe *et al.*, 2003). However, glucose is used to routinely address the growth of many anaerobic bacteria (Grabowski *et al.*, 2005).

At the 17th day of the enrichment assay, the enriched cultures from each reactor were separately re-inoculated in 100% of fresh culture medium (10% of inoculum in culture medium) to remove further components from the original wastewater. Growth profiles observed for all reactors after both re-inoculations were similar to those observed in Figure 4 for reactor A (data not shown). These results demonstrated that all microbial communities were able to adapt to the culture medium addressed despite the different adaptation in the first inoculation. However, lactate was found to be slightly better than glucose in these subsequent re-inoculations. Even though the microbial growth attained was fairly independent of the approach followed, the most common enrichment approach reported in literature is the inoculation of centrifuged biomass (Almeida *et al.*, 2013). Therefore, the community attained in reactor D was selected as inoculum in the experiments described in the next sections.

1.2 Variation of acenaphthene concentration

Haritash and Kaushik (2009) reported that the pre-exposure of a microbial community to hydrocarbons is one of the factors determining the rate of acenaphthene degradation. Adaptation leads to the increase in the hydrocarbon oxidizing potential of the community. Therefore, acenaphthene at a concentration of 100 µg/L was added in all reactors at time 0 to ensure the selectivity pressure during the enrichment. Whenever the concentration of this compound in one reactor was lower than 20 µg/L, a new spike of acenaphthene was carried out to achieve 100 µg/L in all reactors. The variation of the concentration of acenaphthene in all reactors addressed is depicted in Figure 5.

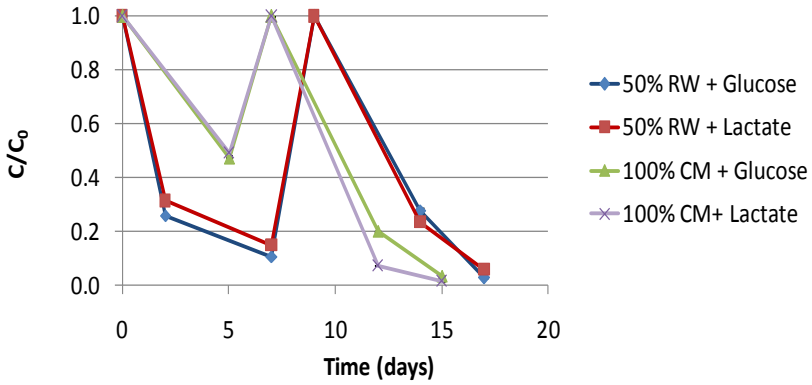


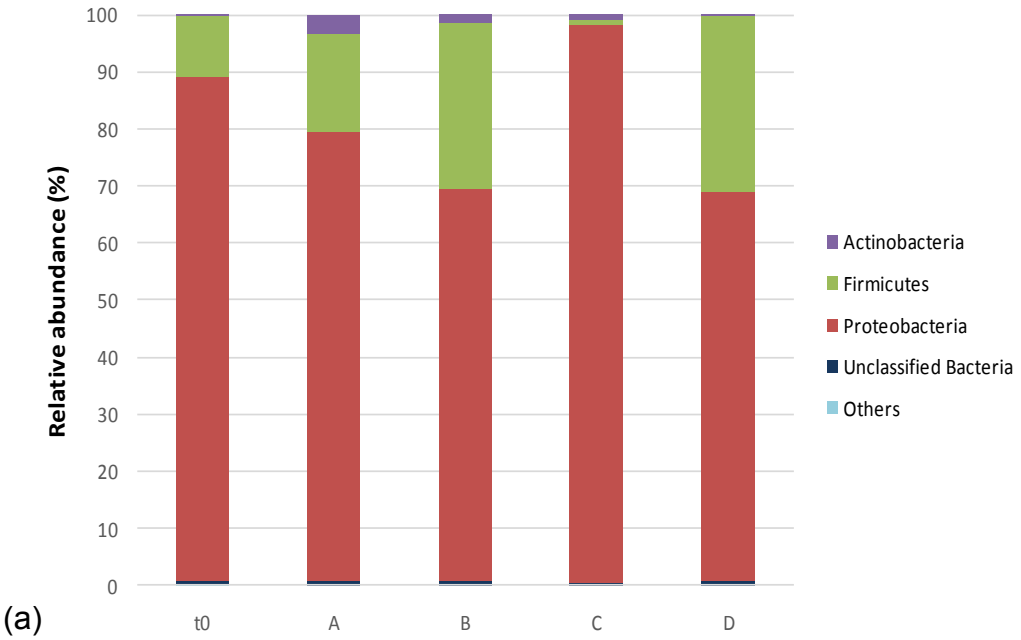
Figure 5 - Variation of C/C_0 of acenaphthene in reactors A to D. RW: Refinery Wastewater; CM: Culture Medium. Were done the following spikes: 100 µg/L of acenaphthene at t_0 and 200 µg/L of acenaphthene at t_9.

Figure 5 shows that acenaphthene concentration decreases over time in all reactors. Furthermore, the concentration determined by HPLC was generally lower than 100 µg/L in the samples taken immediately after spikes conducted at time 9 days for reactors A and B, and time 7 days for reactors C and D. These results indicate that the compound might adsorb to the glass due to its high logarithm of octanol-water partition coefficient (3.92) (ChemSpider) and/or not be very stable since it is a low molecular weight PAH (Lorenzi *et al.*, 2011). Further discussion on this topic will be provided in section 2 and 3 of discussion.

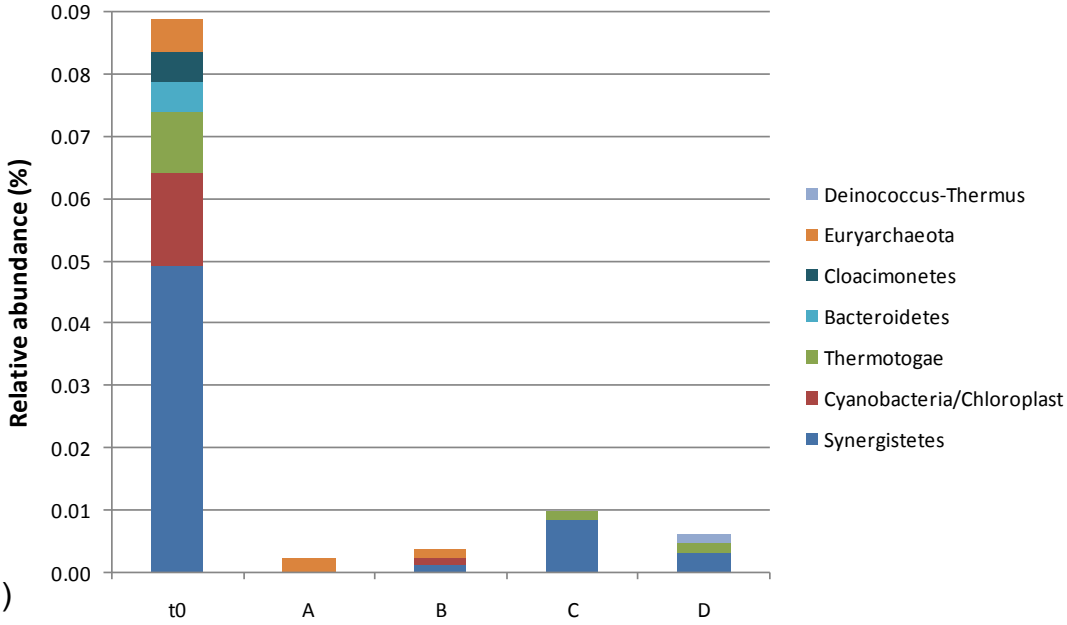
1.3 Characterization of microbial communities

Next-generation sequencing was carried out to address the differences in the microbial communities at time 0 (original refinery wastewater used as inoculum) and in the end of the first inoculation in reactors A to D, during enrichment. All data from sequencing related to enrichment assay are presented in Tables A1 to A5 in Appendix.

Figure 6 and Figure 7 depict the relative abundances attained in terms of phylum and class taxonomic distribution.



(a)



(b)

Figure 6 – Relative abundance of microbial communities at time 0 and in reactors A to D in the end of the first enrichment in terms of phylum: (a) global distribution and (b) distribution of the less abundant [(designed as “others” in figure (a)]. (A) 50% of refinery wastewater and glucose, (B) 50% of refinery wastewater and lactate, (C) 100% of culture medium and glucose, (D) 100% of culture medium and lactate.

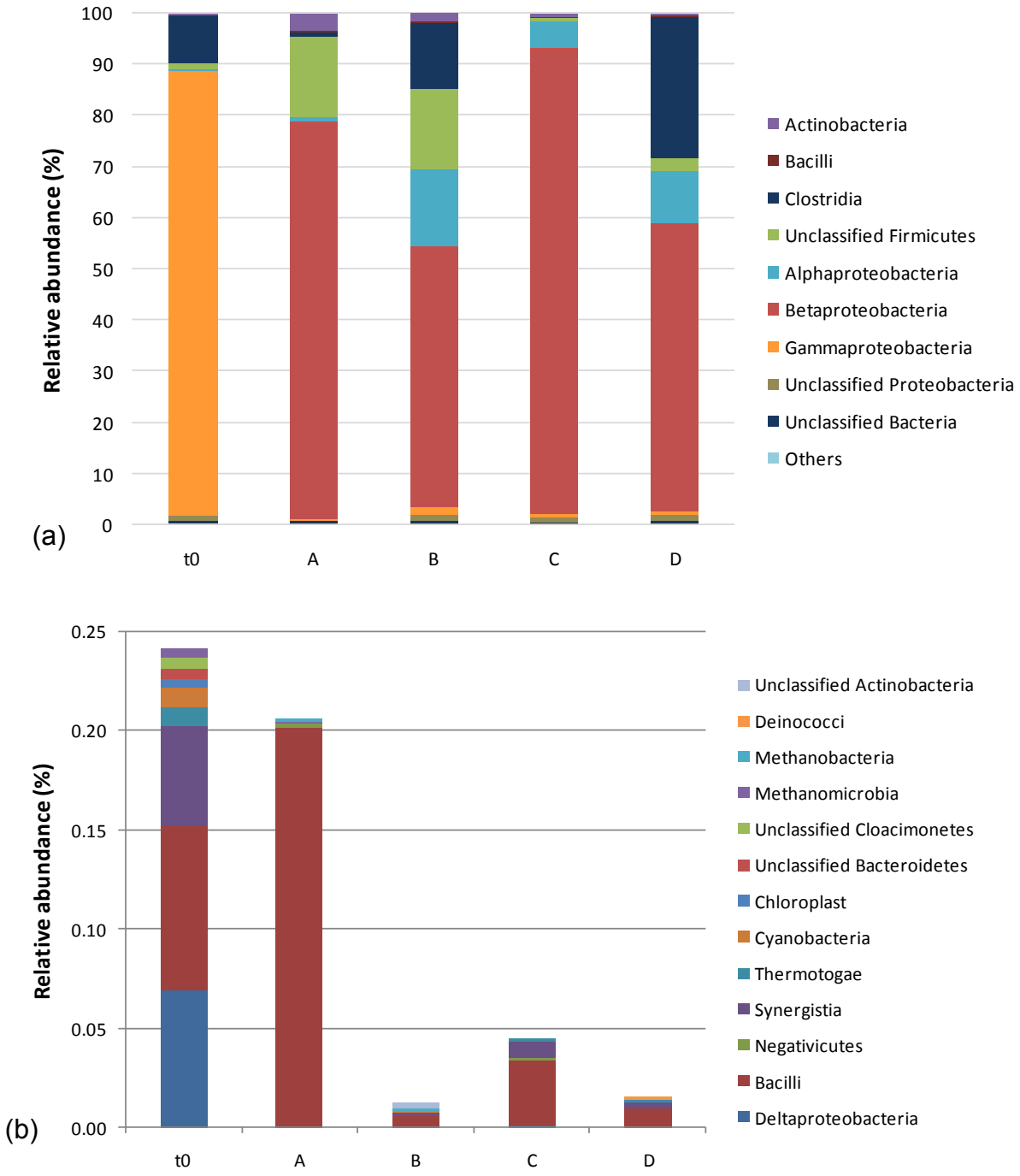


Figure 7 – Relative abundances of microbial communities at time 0 and in reactors A to D in the end of the first enrichment in terms of class: (a) global distribution and (b) distribution of the less abundant [(designed as "others" in figure (a))]. (A) 50% of refinery wastewater and glucose, (B) 50% of refinery wastewater and lactate, (C) 100% of culture medium and glucose, (D) 100% of culture medium and lactate.

The microbial community originally present in the refinery wastewater (time 0) is mainly composed of bacteria from phyla *Proteobacteria* (89%) and *Firmicutes* (11%). *Gammaproteobacteria* class represents 87% of the total abundance and includes mostly bacteria from the *Moraxellaceae* family, which major representatives are Unclassified *Moraxellaceae* (19%) and bacteria from *Acinetobacter* genus (9%) (Table A1 in Appendix). Although bacteria from *Acinetobacter* genus are strictly aerobic (Li *et al*., 2015), have been reported in refinery effluents, as well as *Moraxella* sp., the type genus of *Moraxellaceae* family (Mazzeo *et al*., 2010). Bacteria from this genus cannot make fermentation in the absence of O_2, and were expected to disappear during the anaerobic

enrichment, however, the oxygen production by anaerobic bacteria in its metabolism can explain *Acinetobacter* sp. and Unclassified *Moraxellaceae* presence (Silva *et al*., 2013).

Regarding *Firmicutes*, *Clostridia* class represents 10% of the total abundance and it is essentially composed of microorganisms from *Acetoanaerobium* genus (7%). The other phyla (*Actinobacteria*, *Synergistetes*, *Cyanobacteria/Chloroplast*, *Thermotogae*, *Bacteroidetes*, *Cloacimonetes* and Unclassified *Bacteria*) represent less than 0.6% of the microbial population (Figure 7 and Appendix 1). Microorganisms from the *Archaea* domain, particularly phylum *Euryarchaeota,* were also identified at a relative abundance of 0.005%. Many authors have reported the occurrence of bacteria from phylum *Bacteroidetes* in oil-polluted environments. According with Grabowski *et al*. (2005), the strain BN3^T of *Petrimonas sulfuriphila*, which is member of the same phylum, was isolated from an oilfield. Bacteria from phyla *Actinobacteria* and *Thermotogae* have been also reported in coal deposits and oil reservoirs (Meslé *et al*., 2013). However, it is yet unclear how *Actinobacteria*, which are mainly cellulolytic under aerobic conditions, are able to grow in these environments (Meslé *et al*., 2013), whereas *Thermotogae* scarce abundance could be explained by many members of this phylum growing in anaerobiosis through fermentation (Silva *et al*., 2013). *Synergistetes* phylum was recently reported in samples of crude oil in China (Silva *et al*., 2013). These bacteria are known as aminoacid fermenters, as well as *Cloacimonetes*, both of them able to transform aminoacids in methane in sintrophy with hydrogenotrophic methanogens. *Cloacimonetes* have been detected in wastewaters, namely the candidate division WWE1 (Waste Water of Evry 1), besides bacteria from phylum *Cloacimonetes* have never been isolated, probably because these are symbiotic microorganisms (Chojnacka *et al*., 2015). *Cyanobacteria/Chloroplast* bacteria are described in many industrial effluents such as refinery effluents. When present, this is the dominant phylum between the algae, what is related with the shortage of oxygen and the presence of moderate levels of nutrients (Vijayakumar, 2012) in this type of effluents.

Bacteria from *Proteobacteria* and *Firmicutes* phyla were still the major representatives of the microbial communities in all reactors after the enrichment, showing that these microorganisms were generally able to adapt to the enrichment conditions. However, a noteworthy shift in *Proteobacteria* classes was observed since *Gammaproteobacteria* (87%) and *Betaproteobacteria* (51-91%) were major representatives at time 0 and in the end of the enrichment, respectively [(Figure 7(a))]. Furthermore, *Betaproteobacteria* are present in all the reactors, being more abundant with glucose as carbon source (51% in reactor B and 56% in reactor D), while lactate is a better carbon source to *Clostridia* [(Figure 7(a))]. Furthermore, the major *Betaproteobacteria* – *Diaphorobacter* genus – is much more abundant in the reactors containing glucose (75% and 89% in reactors A and C, respectively, vs 50 and 54% in reactors B and D, respectively) (Table A2 to A5 in Appendix). Bacteria from this genus were detected in sludge from an wastewater treatment plant of a nitroaromatics manufacturing chemical industry (Sing and Ramanathan, 2013). *Diaphorobacter* sp. utilizes yeast extract to grow, which was a component of the culture medium used in the enrichment described in this thesis, although in a very low concentration (0.2 g/L). Furthermore, *Diaphorobacter* sp. can grow under anaerobic conditions and in the presence of nitrate, which was used as electron acceptor (Khan and Hiraishi, 2002). This may explain the predominance of this bacteria genus in reactors A to D. In previous studies, *Diaphorobacter* sp. was also reported in refinery wastewater treatment plants as one of the most abundant genus (Silva *et al*., 2012). In addition, the functional analysis of the metagenome of the bacterial community indicated the presence of genes linked to nitrate and nitrite ammonification and denitrification (Silva *et al*., 2012).

Unclassified *Firmicutes* are likely to have experienced more difficulties to adapt to the new conditions since their relative abundance is higher in reactors containing 50% of refinery wastewater (reactors A and B). Bacteria from *Tissierella* genus are an example of this, since their relative abundance in the presence of lactate was higher in reactors containing 50% of refinery wastewater (8% vs 0.7%). Furthermore, the abundance of *Acetoanaerobium*

genus was reduced from 7% to 0.001% (reactor A) or 0% (reactors B to D), showing that these bacteria were not favored by enrichment conditions. Since, according to Sleat *et al*. (1985) these microorganisms use yeast extract and glucose as carbon sources, other nutrients for their growth could be necessary.

Regarding bacteria present as minority, a reduction in diversity was observed in all reactors during enrichment and their abundances were lower than 0.09% in all reactors [(Figure 6(b))]. Bacteria from phylum *Deinococcus-Thermus* were only detected in reactor D. This phylum is described in sea-water processing wastewater treatment plants, however varies significantly in abundance depending on the wastewater tested (Sanchez *et al*., 2011). Members of *Archaea* domain, which produces methane by acetate or H_2 comsumption, have been reported not only in formation waters (Silva *et al*., 2013), but also have been reported as predominant in produced water, between them *Methanobacterium* sp. (56%), where was verified that the methane production was in agreement with the disappearing of more than 50% of the hydrocarbons, which aromatic used were methylnaphthalene, dimethylnaphthalene, phenanthrene and methylanthracene (Berdugo-Clavijo and Gieg, 2014). These data show that the growth of microorganisms from the *Archaea* domain was not favored by the enrichment conditions because the culture medium used had glucose or lactate and yeast extract, which are more complex carbon sources than acetate or H_2.

Figure 6 and Figure 7 show that the different enrichment strategies in terms of inoculation did not impact significantly communities profiles although changes were observed comparatively with t_0.

2. Resistance assays

The objectives of resistance assays were to test the toxicity of acenaphthene to the microbial community and test the stability of acenaphthene in the culture medium. The concentrations addressed were chosen based on the solubility of acenaphthene in water [(3.9 mg/L (Cerniglia, 1992)] as well as on the occurrence concentrations in refinery wastewaters reported in literature that varied between less than 0.05 µg/L (Benyahia, Abdulkarim and Embaby, 2006) and 606 µg/L (Philemon and Benoît, 2013). The objective in the assessment of concentrations above these values (1000 and 1500 µg/L) was to mimic real conditions and ensure complete solubility of acenaphthene, limiting its adsorption to the reactor walls.

2.1 Microbial growth

Figure 8 shows the growth of the culture in the presence of different concentrations of acenaphthene, where it is represented not only the bacterial growth over the 8 days, but also the optical density of the reactors without cells at different concentrations of acenaphthene (abiotic controls).

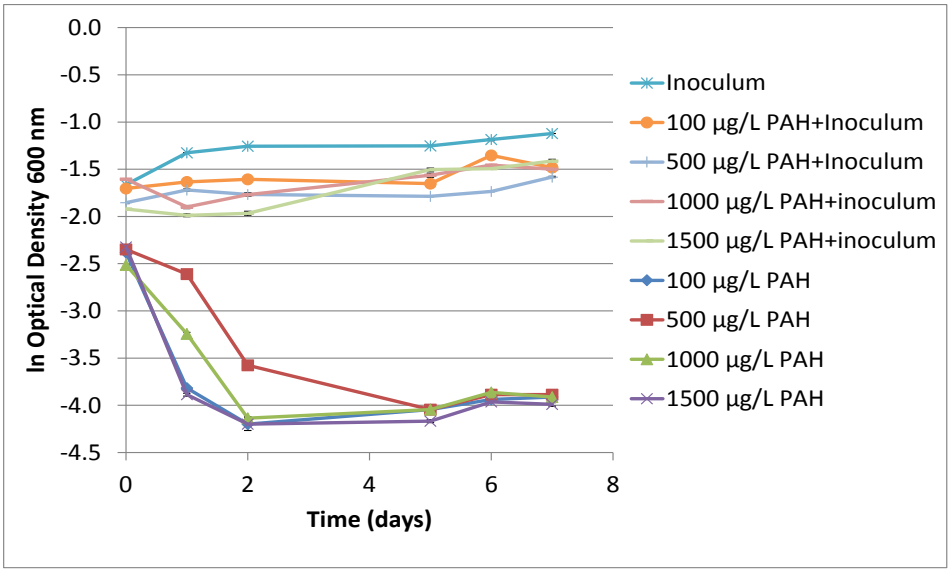


Figure 8 - Logarithm nepperian of the optical density determined at 600 nm during the resistance assays in the reactors containing inoculum as well as in the respective abiotic controls at different acenaphthene concentrations (100, 500, 1000, and 1500 µg/L).

The culture medium prepared for this assay presented a brown colour, which could be related with an excess of iron. During the experiment, the colour of the culture medium in the control reactors got more transparent, which might explain the decrease of the optical density over time in these reactors. Reactions between the iron and the reducing agents may have occurred and might explain these differences in color and optical density.

2.2 Assessment of acenaphthene removal

The variation of acenaphthene concentration as C/C_0 in the different reactors is shown over time in Figure 9.

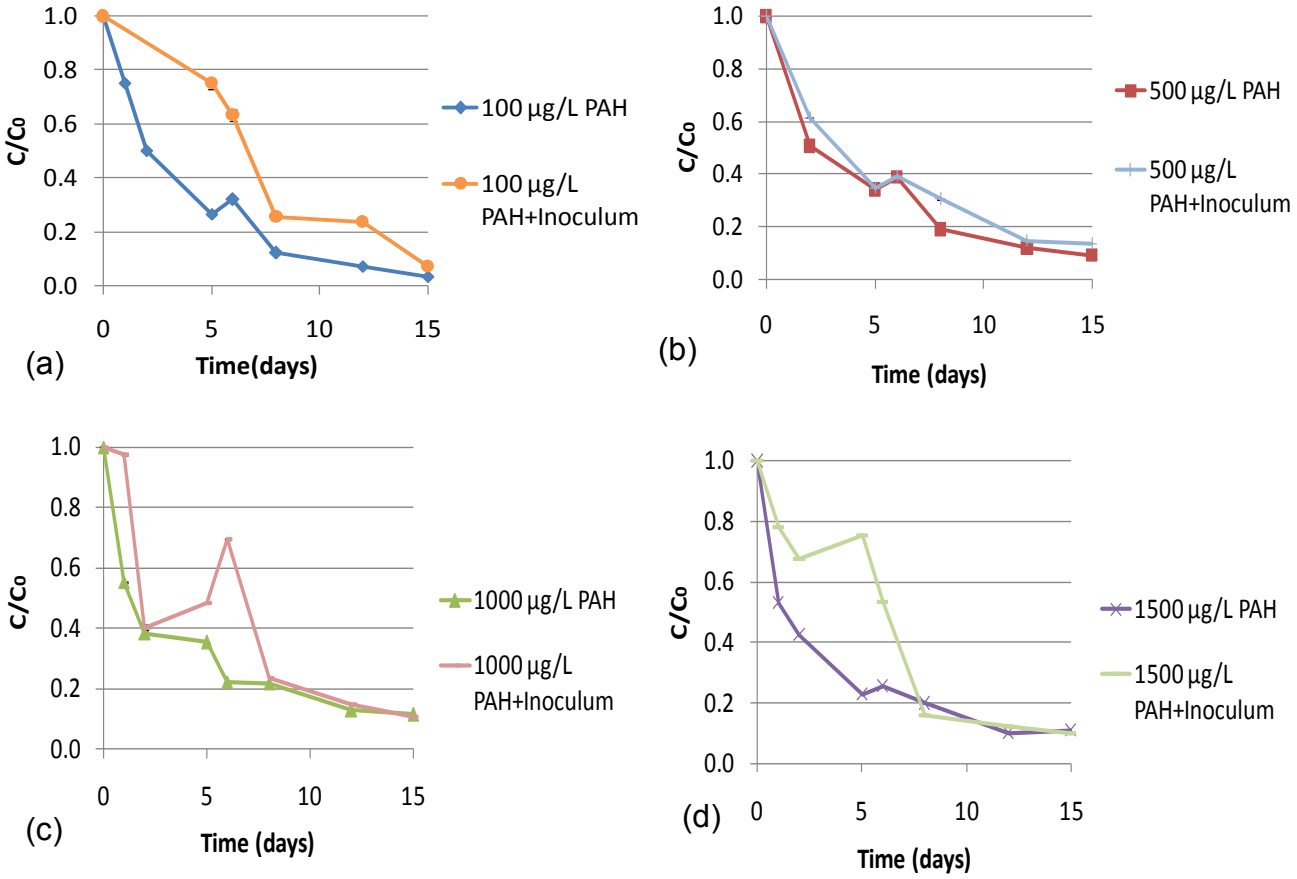


Figure 9 – Variation of acenaphthene concentration (C/C_0) in the reactors containing inoculum as well as in the respective abiotic controls at different acenaphthene concentrations (100, 500, 1000, and 1500 µg/L).

At the different concentrations tested the obtained values representing the removal of acenaphthene were similar. Furthermore, the similar removal of acenaphthene in the absence and presence of the inoculum, particularly in the end of the experiment [(Figure 9(a) to (d))], shows that its removal is probably related with its instability as previously discussed in section 1.2. Tsai, Kumar and Lin (2009) tested fluorene and phenanthrene biodegradation by a mesophilic sulfate reducing bacterial culture, with lactate as carbon source, isolated from anaerobic swine wastewater sludge, and observed adsorption in the abiotic controls, comproving that PAHs are very unstable. Furthermore, Edwards *et al*., (2011) reported that total petroleum hydrocarbons at a concentration of 1.9 mg/L, had a small half-life mainly due to variables like evaporation, photo-oxidation and physical dispersion than due to biodegradation. Combining these results with the fact that the lowest concentrations addressed are more realistic in terms of occurrence in refinery wastewaters and have a lower impact on microbial growth, the concentration of 100 µg/L was selected for the biodegradation studies described in section 3.

3. Biodegradation assay

The objective of this assay was to evaluate acenaphthene biodegradation in the presence and absence of an extra carbon source, in this case lactate, by the reasons mentioned in section 1. The capacity of the microbial community to use acenaphthene as sole carbon source was assessed when lactate was not added to the medium. Microbial growth rates and the community profiles were compared to characterize the microbial population and to evaluate which members of the microbial population could be the most probable candidates of being enrolled in the degradation of the compound. Reactors were prepared and were codified as: (A) PAH + Inoculum + Lactate, (B) PAH + Lactate, (C) PAH + Inoculum, (D) PAH and (E) Inoculum in culture medium without lactate.

3.1 Microbial growth

Figure 10 shows the microbial growth in the presence and absence of the carbon source.

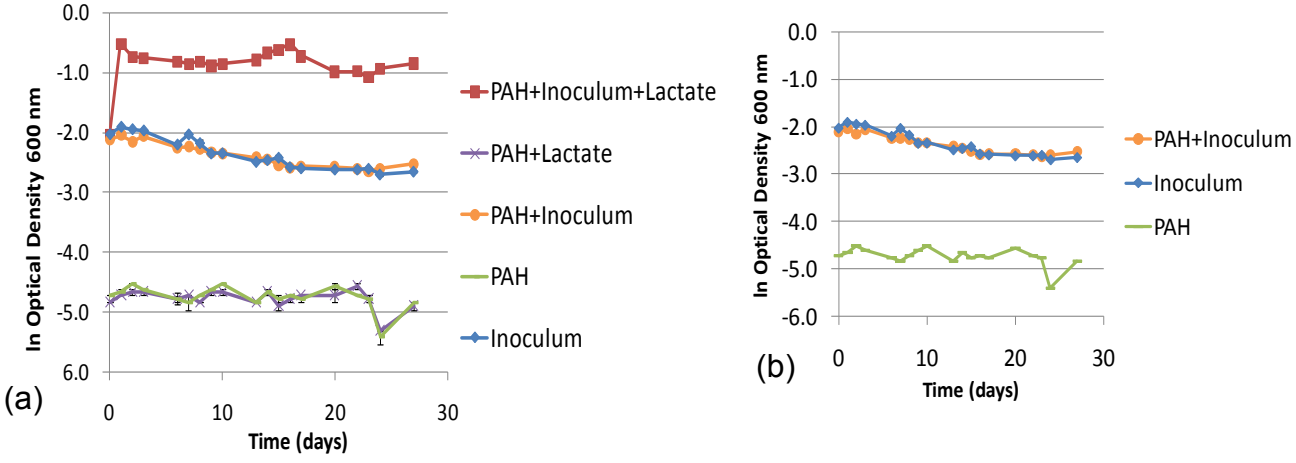


Figure 10 –Microbial growth curves attained at 600 nm: (a) in reactors A to E; (b) in reactors C to E.

Figure 10(a) shows that, in the presence of lactate, the exponential phase is very short, and the microbial community grows very fast, reaching optical densities higher than the ones attained without the supplementation of lactate. In the negative controls (reactor B and D), the optical density was always constant and very low, which was expected since the inoculum was not added in these reactors and acenaphthene doesn't absorb at 600 nm.

In the experiments using acenaphthene as only carbon source [(Figure 10(b))], an exponential phase was not noteworthy. The slight increase in the growth of bacteria in the absence of lactate during the initial 24 h period was

probably due to the residual amount of yeast extract present in the medium. These results suggest that lactate is easier to metabolize by bacteria than acenaphthene. In fact, acenaphthene is an aromatic compound with a more difficult structure to breakdown. Majumder *et al*. (2014) determined that after degradation of hydrocarbons present in a refinery wastewater, aromatic compounds were found in higher concentrations than aliphatic compounds.

The growth observed in the presence of lactate during the biodegradation assay was faster than the growth observed during the enrichment phase (See Figure 4) since, according with data attained in the laboratory, the absence of the reducing agents (trisodium citrate, ascorbic acid, sodium thioglycolate) in the medium favors the growth of this microbial community. Therefore, some of these agents may have some inhibitory effect on the growth of some bacteria.

3.2 Assessment of acenaphthene removal

The concentration of acenaphthene was followed over a 31 days assay to assess the ability of the microbial community to biodegrade acenaphthene. The variation of the concentration of acenaphthene is depicted in Figure 11.

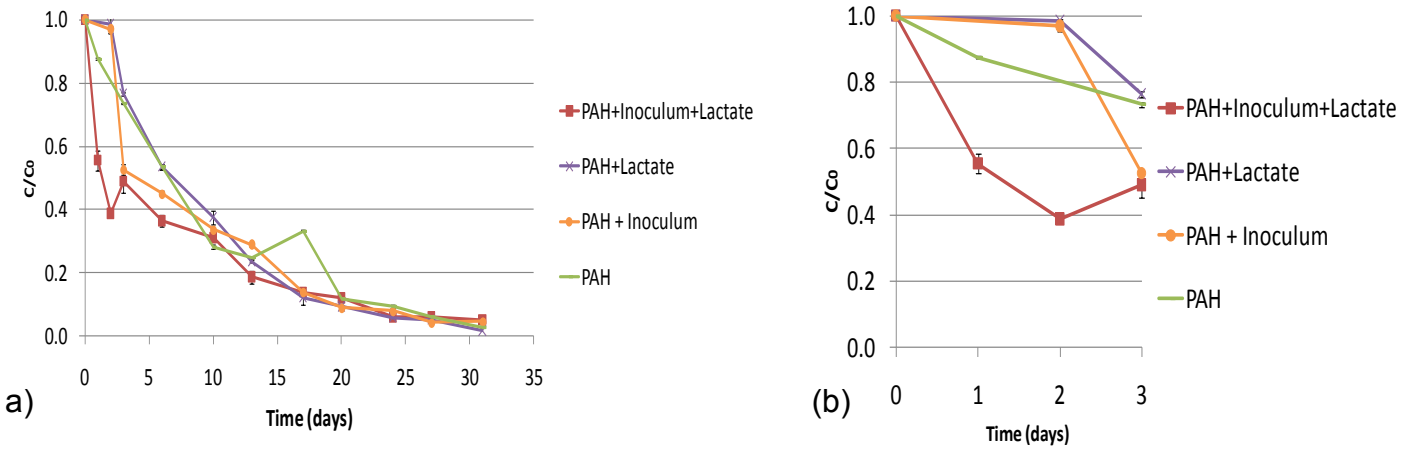


Figure 11 – Variation of the concentration (C/C_0) of acenaphthene: (a) in reactors A to D (b) in reactors A to D from t_0 to t_3 of the assay.

Figure 11(a) shows that, as observed in the enrichment assay (see section 1.2), acenaphthene concentration decreases over time in all reactors, even in the absence of the inoculum. This decrease is particularly pronounced during the initial 10-15 days period. These data is in accordance with data shown in the previous sections of the Results and Discussion chapter (Figure 5 and Figure 9), supporting the instability of acenaphthene in controls that results in similar removals in the presence and absence of the microbial community. Nevertheless, the removal of acenaphthene was slightly faster in the presence of lactate in the initial stage of the experiment (initial 3 days), since with lactate as carbon source (Reactor A) the percentage of remotion hits a maximum of 61% at t_2, whereas with acenaphthene as only carbon source (Reactor C) the percentage of remotion hits a lower maximum (47%), only at t_3. By another hand, in the controls the maximum percentage of remotion it is very similar, which is of 24% in Reactor B and of 27% in reactor D, both of them hitting the maximum at t_3, what shows that lactate does not influence the adsorption of acenaphthene. From the 13rd day of the assay the percentage of remotion it is similar in all reactors. These data suggest that bioremoval might have occurred at some extent either because of the production of cellular machinery to degrade the compound or due to the adsorption of acenaphthene to the microorganisms when microbial population reached a certain concentration (after 24 h). A combination of both mechanisms could also have occured. This feature also explains the higher removal of acenaphthene in the presence of lactate (Reactor A), where the microbial growth is much more pronounced as previously discussed.

A study done with phenanthrene, where a culture was isolated from the anaerobic sludge of swine wastewater, reported that the compound disappeared by biodegradation or biosorption, with the highest percentage of biosorption in the biotic reactor at the highest initial concentration of phenanthrene. The authors concluded that highest the concentration of the PAH, highest is the biosorption (Tsai *et al*., 2009). In another study where phenanthrene was used as a model PAH, it was analyzed how much biosorption contributes to biodegradation. The main conclusion was that PAH biosorption may be important for wastewater refinery treatment systems (Stringfellow and Alvarez-Cohen, 1999).

When exposed to PAHs, bacteria biodegraders amplify genes involved in acenaphthene metabolism which according with Haritash and Kaushik (2009), can occur through selective enrichment or gene transfer. On the other hand, the addition of an extra carbon source may enhance the biodegradation ratio by stimulating the growth of biodegraders microorganisms, known as 'biostimulation', which was already reported for lactate, explaining the slightly higher degradation observed in its presence in this study (Haritash and Kaushik, 2009).

As an attempt to better understand the removal of acenaphthene in the presence and absence of the microbial community, the Estimation Programs Interface (EPI Suite)™, developed by the Environmental Protection Agency, was used. This program estimates physical and chemical properties of several organic compounds as well as their environmental fate properties, which can indicate where a chemical can be found in the environment and how long it is expected to remain there. PAHs have many ways of dispersion such as volatilization, photo-oxidation, chemical oxidation, adsorption on sediments and microbial degradation (Haritash and Kaushik, 2009). According with BIOWIN 7, the Anaerobic Probability Model used by EPI Suite, the probability of acenaphthene to be biodegraded under anaerobic conditions is very low – 0.0199, which is relatively in line with data shown in Figure 11 for the reactors containing the microbial community.

Table 6 show the fugacity of acenaphthene in air, water, soil, and sediments in terms of distribution and half-life, according with the Level II Fugacity Model integrated in the EPI Suite.

Table 6 - Distribution of acenaphthene in air, water, soil and sediments.

	Mass Amount (%)	**Half Life (hours)**
Air	0.278	4.43
Water	11.7	900
Soil	84.6	$1.8x10^3$
Sediment	3.4	$1.8x10^3$

Data shown in Table 6 show that acenaphthene is expected to strongly adsorb on soil and sediments and is, therefore, expected to be adsorbed on the glass walls of the reactors. Combining these data with the previous discussion regarding the high log K_{ow} of acenaphthene, the removal of this compound in the negative control might be due to its ability to rather adsorb to other elements than remain soluble in water. This also corroborates the fact that the concentration determined after each spike was lower than the concentration effectively expected, even in the negative controls. Looking at the compound half-life in water (900 h; approximately 38 days) as well as the removal of acenaphthene by the microorganisms in the initial stages of the process [(Figure(b))], a further optimized anaerobic biodegradation process could be advantageous in case residential times in wastewater treatment plants are not long.

The results attained show that, despite the significant acenaphthene removal due to its instability in water, this compound is still a problem because of its persistence since it remains in sediments of treated effluents. Chemical processes could be better treatment options since the accessibility of the compound to microorganisms (due to high adsorption and instability) would not be a problem.

3.3 Characterization of microbial communities

Microbial population was characterized by next-generation sequencing at time 0 (inoculums selected after the microbial enrichment described in section 1.1 and that was used in the resistance assays) and in the end of the biodegradation assay (day 31) in reactors A (reactor containing lactate) and C (reactor without lactate). All data from sequencing related to biodegradation assay are presented in Tables A6 to A8 in Appendix. Figure 12 depicts the relative abundance attained in terms of global abundance [(Figure 12(a))] and relative abundance of the minority phyla [(Figure 12(b))].

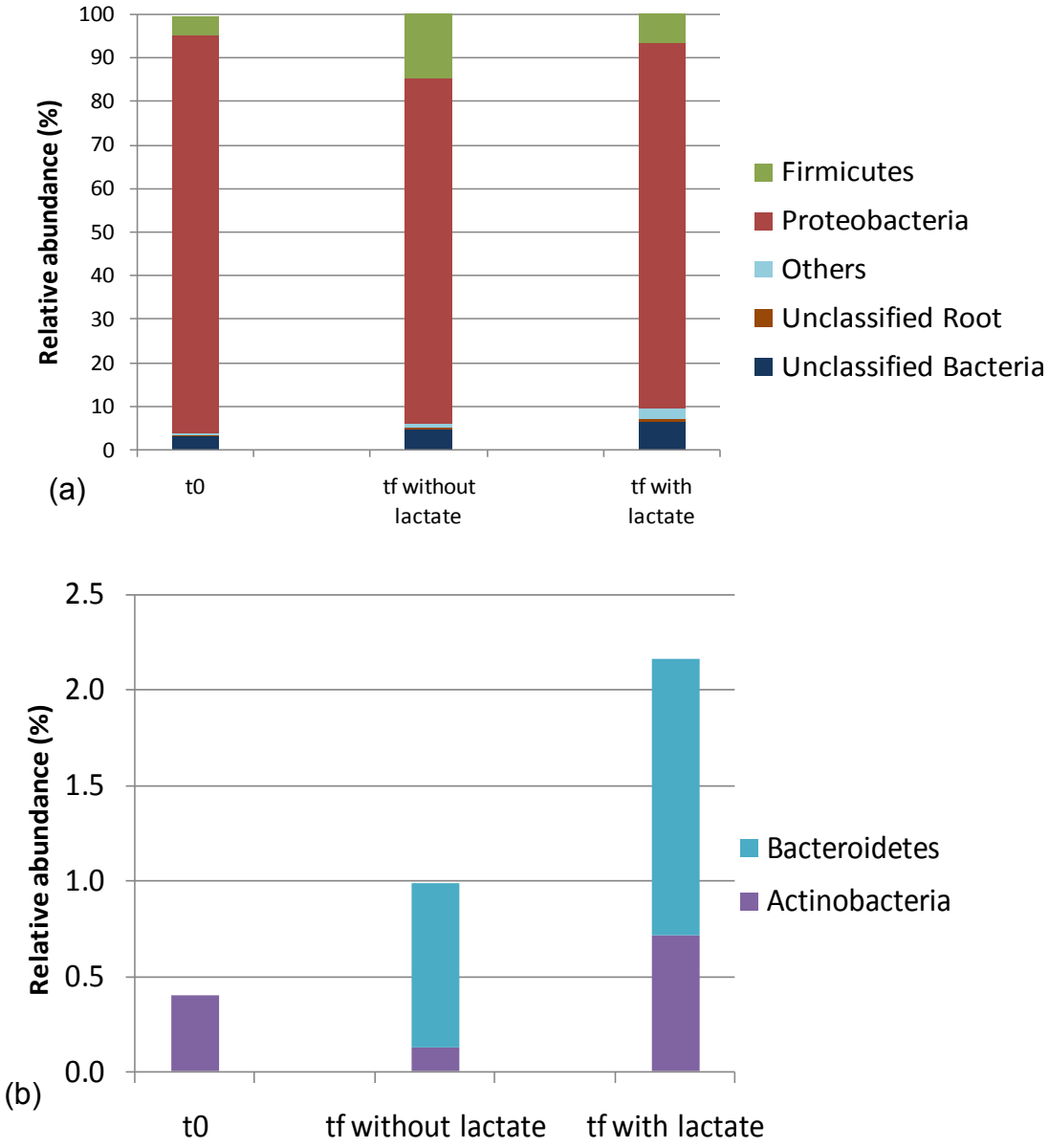


Figure 12 - Relative abundance of microbial communities at time 0 (t_0) and in the end of the biodegradation assay (t_f) in the reactors without lactate (reactor C) and with lactate (reactor A) in terms of phylum: (a) global distribution and (b) distribution of the less abundant [(identified as "others" in Figure 12(a)].

Figure 13 depicts the relative abundances attained in terms of global abundance [(Figure 13(a))] and relative abundance of the minority classes [(Figure 13(b))], respectively.

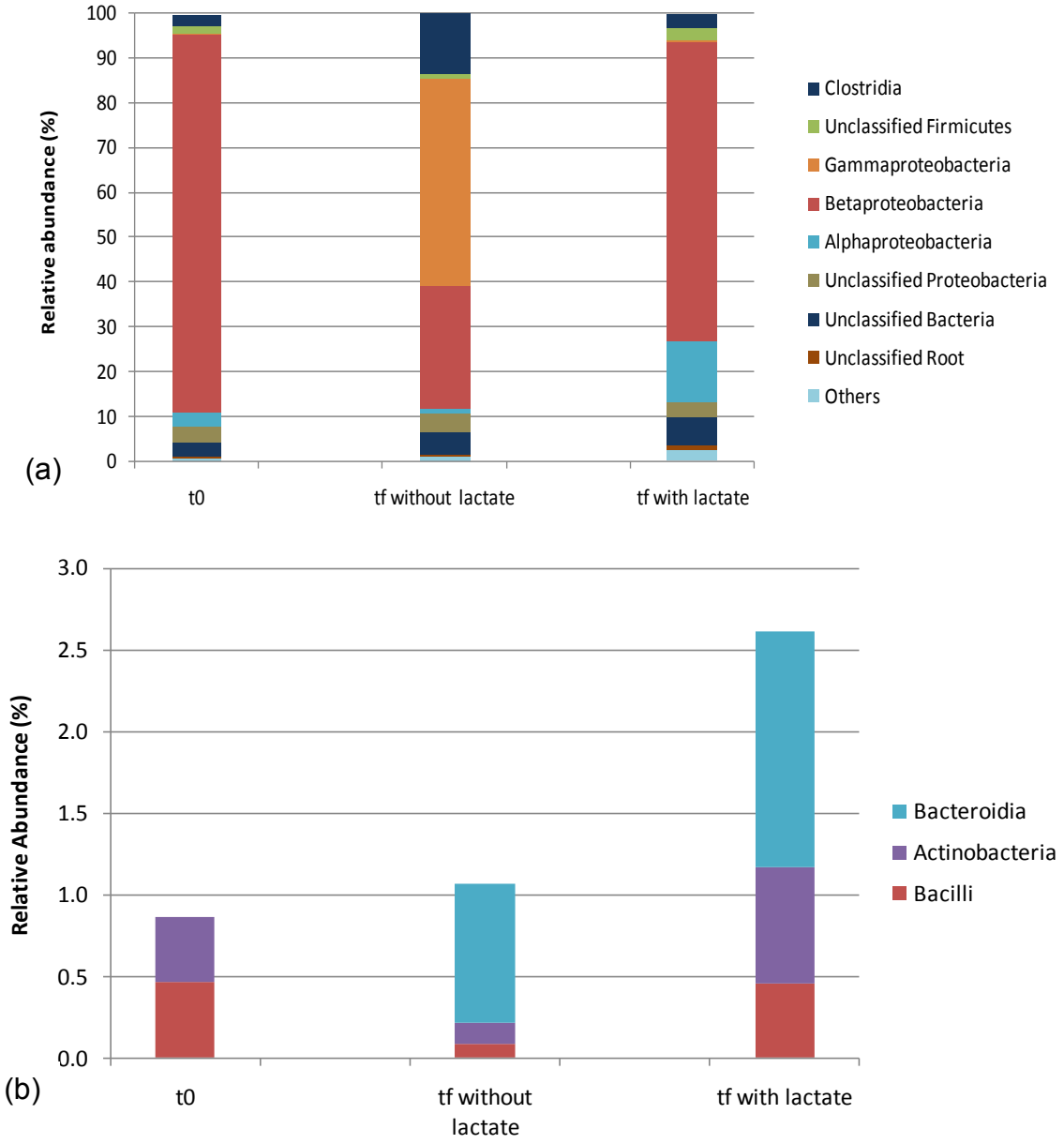


Figure 13 - Relative abundance of microbial communities at time 0 (t_0) and in the end of the biodegradation assay (t_f) in the reactors without lactate (C) and with lactate (A) in terms of class: (a) global distribution and (b) distribution of the less abundant [(identified as "others" in Figure 13(a))].

At the initial time (t_0), the abundance of the major phyla is similar to the one determined in the reactor D in the end of the enrichment, with 91% of *Proteobacteria*, and 5% of *Firmicutes* [(Figure 7(a) and Figure 12(a))], which was expected. The relative abundances of the major phyla in the reactor containing lactate are slightly more similar to the ones that correspond to time 0 than with the reactor without lactate. This higher similarity was expected since the composition of the medium in these reactors was not changed. The differences in the microbial profile of the inoculum used in this assay and the one attained for the reactor D in the end of the enrichment (Figure 7) is probably due to the changes carried out in terms of the withdrawal of the reducing agents from the culture medium, as previously described. Figure 12(b) and Figure 13(b) also show that the diversity of the less abundant phyla is low.

Figure 13(a) shows that the presence of lactate highly impacts the abundance of some classes present in the reactors. At time 0, *Proteobacteria* phylum is mainly constituted by bacteria from the classes *Betaproteobacteria* (85%) and *Alphaproteobacteria* (3%), whereas *Gammaproteobacteria* are the minor class of this phylum (0.1%). The predominance of *Betaproteobacteria* in the presence of lactate is mainly due to Unclassified *Betaproteobacteria* (35%), *Diaphorobacter* sp. (24%), and *Thauera* sp. (11%) (Table A7 in Appendix). *Proteobacteria* abundance was influenced by the presence of the extra carbon source (lactate): with lactate, the major classes are *Betaproteobacteria* and *Alphaproteobacteria* while *Gammaproteobacteria* is the major class when acenaphthene is the only carbon source, although *Betaproteobacteria* are also present. The most abundant *Betaproteobacteria* in the presence of lactate is *Diaphorobacter* sp. (21%), which is similar to its abundance in the beginning of the assay (24%) and slightly less abundant in the absence of lactate (12%). Commonly, this bacteria uses aminoacids (α-alanine and α-asparagine), yeast extract, peptone and also ethanol, acetate and pyruvate as carbon sources (Khan and Hiraishi, 2002). A common supplement used in *Diaphorobacter sp.* growth is yeast extract, which is present in much less quantity than during the enrichment phase and may contribute to the decrease in relative abundance relatively to the values indicated above (Khan and Hiraishi, 2002). *Diaphorobacter* sp. has already been reported to use nitroaromatic compounds as sole source of carbon, nitrogen and energy, with nitrite release (Singh and Ramanathan, 2013) and, in fact, nitrate is present in the culture medium used in the study reported in this thesis. For all these reasons, *Diaphorobacter* sp. may be involved in acenaphthene biodegradation.

Thauera sp. relative abundances are nearly the same at time 0 and in the reactor with lactate (10% and 8% respectively), whereas in the reactor without lactate its relative abundance is 2%. It is known that *Thauera* sp. grows under anaerobiosis with selenate or nitrate as electron acceptor and that one of the carbon sources that it is able to use is lactate, which justify the higher relative abundance in the presence of lactate (Macy *et al*., 1993). Since *Thauera* sp. is more abundant in the presence of lactate, it may also be involved in acenaphthene removal. In fact, *Thauera* sp. is reported as a biodegrader of a lot of alkylbenzenes, as toluene (Meckenstock, Safinowski and Griebler, 2004), which metabolic pathway was suggested by Heider *et al*. (1999).

Gammaproteobacteria are present only in the reactor without lactate, what is mainly due to relative abundance of Unclassified *Gammaproteobacteria* (31%) and *Acinetobacter* sp. Bacteria from *Acinetobacter* genus are strictly aerobic (Li *et al*., 2015), so the only explanation for its presence it is the oxygen production by metabolism of anaerobic bacteria (Silva *et al*., 2013). Furthermore, since *Acinetobacter* sp. relative abundance decreases in the reactor with lactate, probably is not responsible for acenaphthene degradation.

Firmicutes phylum mainly includes *Proteiniclasticum* sp. (3%) and Unclassified *Firmicutes* (3%), and their low abundance might be explained by the absence of specific nutrients necessary for their growth (Zhang, Song and Dhong, 2010), since their preferred carbon sources are aminoacids instead of lactate or aromatic hydrocarbons (Zhang, Song and Dhong, 2010).

Regarding the minority phyla (*Bacteroidetes* and *Actinobacteria*), these represent less than 4% of the total community. Some *Actinobacteria* have been described in many petroleum reservoirs worldwide and reported as hydrocarbon degraders in aerobiosis (see section 1.3 of discussion) (Silva *et al*., 2013). *Dietzia* sp., for instance, was isolated from Campos Basin and the enzymes responsible by biodegradation of hydrocarbons presented high activity (Silva *et al*., 2013). However, *Streptomyces* spp., another *Actinobacteria*, was detected in an oil Brazilian basin, where biodegradation was not observed, suggesting that not all *Actinobacteria* may be enroled in biodegradation of hydrocarbons in oil fields (Silva *et al*., 2013). *Bacteroidetes* use yeast extract as carbon source (Chen and Dong, 2005) and are widely represented not only in oil reservoirs and coal deposits, being responsible by metabolism of its organic acids (Meslé, Dromart and Oger, 2013), but also in wastewater treatment systems,

having a role in fermentation of organic compounds (Silva *et al*., 2013). Since *Bacteroidetes* are slightly more abundant in the reactor with lactate, this phylum can be involved in acenaphthene biodegradation in some way.

Conclusions and Future work

In this study, the enrichment of microbial communities from a petrochemical refinery wastewater was successfully accomplished, independently of the enrichment strategy followed. Microbial communities were able to adapt to acenaphthene and grow when an extra carbon source – glucose or lactate – were added. However, the microbial community grew faster in the presence of lactate as well as in the absence of reducing agents. Furthermore, the use of lactate led to a higher diversity in the microbial community. The microbial community selected as inoculum for resistance and biodegradation assays was mainly constituted by *Betaproteobacteria*, *Alphaproteobacteria*, Unclassified *Firmicutes* and *Clostridia*. *Betaproteobacteria* were the most abundant, followed by *Clostridia*, *Alphaproteobacteria* and Unclassified *Firmicutes*. The main *Betaproteobacteria* present have been reported in refinery wastewaters (Silva *et al*., 2013) and use nitrate as electron acceptor (Singh and Ramanathan, 2013).

It was also shown that acenaphthene can be toxic for bacteria at concentrations from 500 to 1200 µg/L. Since acenaphthene is generally present at lower concentrations in refinery wastewaters, its toxicity at such concentrations seems not to be a noteworthy problem for bacterial growth. The abiotic controls carried out during the studies performed showed that this compound is very unstable and hydrophobic. Therefore, biodegradation under the addressed conditions may not be suitable for acenaphthene removal unless residence times are short (less than 3 h) in wastewater treatment plants, because it is removed over time even in the abiotic control (27% of remotion at the third day of the assay). Furthermore, the maximum percentage of remotion in the presence of lactate was hitten at day 2 (61%) and at day 3 (47%) in its absence. Otherwise, other treatment processes that could remove acenaphthene faster could be better alternatives since this compound presents high affinity towards sediments and particles and, therefore, it is still a threat for the aquatic environment. It can be also concluded that biodegradation occurs mainly in the presence of an extra carbon source, either because of the need to produce cellular machinery to degrade acenaphthene or because of the need to achieve high cellular density for biosorption.

In further studies, it would be interesting to assess how much the biosorption of acenaphthene contributes for its removal, since due to its high hidrophobicity acenaphthene can adsorb to the reactor walls, be degraded by bacteria or adsorb to biomass. These studies could also have in consideration the adsorption of acenaphthene to other components present in refinery wastewaters, like organic compounds and trace metals.

Other carbon sources, pH, salinity, temperature, among other important factors impacting biodegradation, could be also addressed to improve biodegradation. It could be also interesting to isolate microorganisms from the community to understand the individual role of each one in bioremoval as well as to identify the genes and/or proteins involved in the biodegradation pathway. Another interesting study would be to analyze acenaphthene toxicity along biodegradation.

The ability of the selected microbial community to remove other micropollutants present in refinery wastewaters, particularly compounds that are priority pollutants according to EPA, could also be evaluated.

Another interesting work would be the evaluation of alternative treatments for acenaphthene removal, that could allow overcoming the problems faced during this work in terms of the adsorption and instability of the compound.

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Appendix

Table A1 - Relative abundance and taxonomic characterization of the microorganisms present at time 0 of the enrichment assay.

Lineage	Name	Rank	Nº sequences	Relative abundance (%)
Bacteria				
Root;rootrank;*Bacteria*;domain;unclassified_Bacteria;	Unclassified Bacteria	domain	128	0,631
Proteobacteria		phylum		
Gammaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; Pseudomonadales;order;Moraxellaceae;family;Acinetobacter;genus;	Acinetobacter	genus	1807	8,911
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; Pseudomonadales;order;Moraxellaceae;family;unclassified Moraxellaceae;	Unclassified Moraxellaceae	family	3791	18,695
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; Pseudomonadales;order;unclassified Pseudomonadales;	Unclassified Pseudomonadales	order	53	0,261
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; "Enterobacteriales";order;Enterobacteriaceae;family;Salmonella;genus;	Salmonella	genus	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; "Enterobacteriales";order;Enterobacteriaceae;family;unclassified Enterobacteriaceae;	Unclassified Enterobacteriaceae	family	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Alteromonadales;order; Alteromonadaceae;family;Alishewanella;genus;	Alishewanella	genus	7	0,035
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; *Xanthomonadales*;order;Xanthomonadaceae;family;Stenotrophomonas;genus;	Stenotrophomonas	genus	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; Unclassified Gammaproteobacteria;	Unclassified Gammaproteobacteria	class	11991	59,133
Betaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Diaphorobacter;genus;	Diaphorobacter	genus	3	0,015
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Curvibacter;genus;	Curvibacter	genus	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;unclassified Comamonadaceae;	Unclassified Comamonadaceae	family	20	0,099
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Hydrogenophilales;order; Hydrogenophilaceae;family;Tepidiphilus;genus;	Tepidiphilus	genus	5	0,025
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Hydrogenophilales;order; Hydrogenophilaceae;family; unclassified Hydrogenophilaceae;	Unclassified Hydrogenophilaceae	family	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order; Rhodocyclaceae;family;Thauera;genus;	Thauera	genus	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order; Rhodocyclaceae;family;Azospira;genus;	Azospira	genus	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order; Rhodocyclaceae;family;unclassified_Rhodocyclaceae;	Unclassified Rhodocyclaceae	family	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class; unclassified Betaproteobacteria;	Unclassified Betaproteobacteria	class	4	0,020
Deltaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Deltaproteobacteria;class; Desulfuromonadales;order;unclassified Desulfuromonadales;	Unclassified Desulfuromonadales	order	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Deltaproteobacteria;class; Desulfuromonadales;order;unclassified Desulfuromonadales;	Unclassified Desulfuromonadales	order	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Deltaproteobacteria;class;Desulfovibrionales;order; Desulfomicrobiaceae;family;Desulfomicrobium;genus;	Desulfomicrobium	genus	11	0,054

Table 1 - Relative abundance and taxonomic characterization of the microorganisms present at time 0 of the enrichment assay (continuation).

Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Deltaproteobacteria;class;Desulfuromonadales;order;unclassified Desulfuromonadales;	Unclassified Desulfuromonadales	order	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Deltaproteobacteria;class;Desulfovibrionales;order;Desulfomicrobiaceae;family;Desulfomicrobium;genus;	Desulfomicrobium	genus	11	0,054
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Deltaproteobacteria;class;unclassified Deltaproteobacteria;	Unclassified Deltaproteobacteria	class	2	0,010
Alphaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order;Caulobacteraceae;family;Brevundimonas;genus;	Brevundimonas	genus	9	0,044
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhizobiales;order;Hyphomicrobiaceae;family;Devosia;genus;	Devosia	genus	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhizobiales;order;Methylocystaceae;family;Methylosinus;genus;	Methylosinus	genus	2	0,010
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order;Rhodobacteraceae;family;unclassified Rhodobacteraceae;	Unclassified Rhodobacteraceae	order	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;unclassified Alphaproteobacteria;	Unclassified Alphaproteobacteria	class	1	0,005
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;unclassified "Proteobacteria";	Unclassified" Proteobacteria"	phylum	232	1,144
Firmicutes		phylum		
Bacilli	Bacilli	class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Bacillales;order;Listeriaceae;family;Listeria;genus;	Listeria	genus	7	0,035
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Carnobacteriaceae;family;Trichococcus;genus;	Trichococcus	genus	2	0,010
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;unclassified Lactobacillales;	Unclassified Lactobacillales	order	1	0,005
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;unclassified Bacilli;	Unclassified Bacilli	class	7	0,035
Clostridia	Clostridia	class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Peptostreptococcaceae;family;Acetoanaerobium;genus;	Acetoanaerobium	genus	1347	6,643
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1;family;Proteiniclasticum;genus;	Proteiniclasticum	genus	123	0,607
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1;family;Clostridium sensu stricto;genus;	Clostridium sensu stricto	genus	1	0,005
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1;family;unclassified Clostridiaceae 1;	Unclassified Clostridiaceae 1	family	221	1,090
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Peptostreptococcaceae;family;unclassified Peptostreptococcaceae;	Unclassified Peptostreptococcaceae	family	160	0,789
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiales_Incertae Sedis XI;family;Tissierella;genus;	Tissierella	genus	4	0,020
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 2;family;unclassified Clostridiaceae 2;	Unclassified Clostridiaceae 2	family	3	0,015
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Eubacteriaceae;family;Acetobacterium;genus;	Acetobacterium	genus	5	0,025
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiales_Incertae Sedis XI;family;unclassified Clostridiales Incertae Sedis XI;	Unclassified Clostridiales Incertae Sedis XI	family	1	0,005
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;unclassified Clostridiales;	Unclassified Clostridiales	order	93	0,459
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;unclassified Clostridiales;	Unclassified Clostridiales	order	93	0,459

Table A1 – Relative abundance and taxonomic characterization of the microorganisms present at time 0 of the enrichment assay (continuation).

Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; unclassified Clostridiales;	Unclassified Clostridiales	order	93	0,459
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Thermoanaerobacterales;order; Thermoanaerobacteraceae;family;Caldanaerobacter;genus;	Caldanaerobacter	genus	1	0,005
Root;rootrank;Bacteria;domain;Firmicutes;phylum;unclassified Firmicutes;	Unclassified Firmicutes	phylum	203	1,001
Thermotogae		phylum		
Root;rootrank;Bacteria;domain;"Thermotogae";phylum;Thermotogae;class;Thermotogales;order; Thermotogaceae;family;Petrotoga;genus;	Petrotoga	genus	1	0,005
Root;rootrank;Bacteria;domain;"Thermotogae";phylum;Thermotogae;class;Thermotogales;order; Thermotogaceae;family;unclassified Thermotogaceae;	Unclassified Thermotogaceae	family	1	0,005
Synergistetes		phylum		
Root;rootrank;Bacteria;domain;"Synergistetes";phylum;Synergistia;class;Synergistales;order; Synergistaceae;family;unclassified Synergistaceae;	Unclassified Synergistaceae	family	10	0,049
Cyanobacteria/Chloroplast		phylum		
Root;rootrank;Bacteria;domain;Cyanobacteria/Chloroplast;phylum;Chloroplast;class;Chloroplast;family; Chlorophyta;genus;	Chlorophyta	genus	1	0,005
Root;rootrank;Bacteria;domain;Cyanobacteria/Chloroplast;phylum;Cyanobacteria;class;Family II;family; GpIIa;genus;	GpIIa	genus	2	0,010
Actinobacteria		phylum		
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae; subclass;Actinomycetales;order;Corynebacterineae;suborder;Dietziaceae;family;Dietzia;genus;	Dietzia	genus	1	0,005
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;unclassified Actinobacteria;	Unclassified Actinobacteria	class	3	0,015
Bacteroidetes		phylum		
Root;rootrank;Bacteria;domain;"Bacteroidetes";phylum;unclassified "Bacteroidetes";	Unclassified "Bacteroidetes"		1	0,005
Cloacimonetes	Cloacimonetes	phylum	1	0,005
Root;rootrank;Bacteria;domain;Cloacimonetes;phylum;Candidatus Cloacamonas;genus;	Candidatus Cloacamonas	genus	1	0,005
Archaea	Archaea	domain		
Euryarchaeota		phylum		
Root;rootrank;Archaea;domain;"Euryarchaeota";phylum;"Methanomicrobia";class;Methanosarcinales;order; Methanotrichaceae;family;Methanothrix;genus;	Methanothrix	genus	1	0,005
Total			20278	100

Table A2- Relative abundance and taxonomic characterization of the microorganisms present in the reactor A (50% of refinery wastewater + 50% of synthetic medium + glucose) of the enrichment assay.

Lineage	Name	Rank	Nº sequences	Relative abundance (%)
Bacteria			87902	
Root;rootrank;Bacteria;domain;unclassified_Bacteria;	Unclassified Bacteria	domain	806	0,917
Proteobacteria		phylum		
Betaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Diaphorobacter;genus;	Diaphorobacter	genus	66242	75,357
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;unclassified_Comamonadaceae;	Unclassified Comamonadaceae	family	1900	2,161
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Alcaligenaceae;family;Pusillimonas;genus;	Pusillimonas	genus	10	0,011
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Alcaligenaceae;family;unclassified_Alcaligenaceae;	Unclassified Alcaligenaceae	family	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; unclassified_Burkholderiales;	Unclassified Burkholderiales	order	3	0,003
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order; Rhodocyclaceae;family;Thauera;genus;	Thauera	genus	11	0,013

Table A2 - Relative abundance and taxonomic characterization of the microorganisms present in the reactor A (50% of refinery wastewater + 50% of synthetic medium + glucose)of the enrichment assay (continuation).

Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; unclassified_Burkholderiales;	Unclassified Burkholderiales	order	3	0,003
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order; Rhodocyclaceae;family;Thauera;genus;	Thauera	genus	11	0,013
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order; Rhodocyclaceae;family;unclassified_Rhodocyclaceae;	Unclassified Rhodocyclaceae	family	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Hydrogenophilales;order; Hydrogenophilaceae;family;Tepidiphilus;genus;	Tepidiphilus	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Hydrogenophilales;order; Hydrogenophilaceae;family;unclassified_Hydrogenophilaceae;	Unclassified Hydrogenophilaceae	family	2	0,002
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class; unclassified_Betaproteobacteria;	Unclassified Betaproteobacteria	class	4	0,005
Alphaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order; Caulobacteraceae;family;Brevundimonas;genus;	Brevundimonas	genus	454	0,516
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order; Caulobacteraceae;family;unclassified_Caulobacteraceae;	Unclassified Caulobacteraceae	family	6	0,007
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order; Rhodobacteraceae;family;Rhodobacter;genus;	Rhodobacter	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order; Rhodobacteraceae;family;Paracoccus;genus;	Paracoccus	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order; Rhodobacteraceae;family;unclassified_Rhodobacteraceae;	Unclassified Rhodobacteraceae	family	287	0,326
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhizobiales;order; Methylocystaceae;family;Methylosinus;genus;	Methylosinus	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhizobiales;order; Bradyrhizobiaceae;family;Bradyrhizobium;genus;	Bradyrhizobium	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class; unclassified_Alphaproteobacteria;	Unclassified Alphaproteobacteria	class	29	0,033
Gammaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; Xanthomonadales;order;Xanthomonadaceae;family;Stenotrophomonas;genus;	Stenotrophomonas	genus	35	0,040
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; Xanthomonadales;order;Xanthomonadaceae;family;unclassified_Xanthomonadaceae;	Unclassified Xanthomonadaceae	family	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; Pseudomonadales;order;Moraxellaceae;family;Acinetobacter;genus;	Acinetobacter	genus	9	0,010
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; Pseudomonadales;order;Moraxellaceae;family;unclassified_Moraxellaceae;	Unclassified Moraxellaceae	family	14	0,016
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Legionellales;order; Legionellaceae;family;Legionella;genus;	Legionella	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; unclassified_Gammaproteobacteria;	Unclassified Gammaproteobacteria	class	77	0,088
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;unclassified_"Proteobacteria";	Unclassified "Proteobacteria"		129	0,147
Firmicutes		phylum		
Clostridia		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiaceae 1;family; Proteiniclasticum;genus;	Proteiniclasticum	genus	207	0,235
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiaceae 1;family; unclassified_Clostridiaceae 1;	Unclassified Clostridiaceae 1	order	308	0,350
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiales_Incertae Sedis XI;family;Sedimentibacter;genus;	Sedimentibacter	genus	248	0,282

Table A2 - Relative abundance and taxonomic characterization of the microorganisms present in the reactor A (50% of refinery wastewater + 50% of synthetic medium + glucose)of the enrichment assay (continuation).

Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiales_Incertae Sedis XI;family;Tissierella;genus;	Tissierella	genus	7	0,008
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Eubacteriaceae;family; Acetobacterium;genus;	Acetobacterium	genus	3	0,003
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Peptostreptococcaceae;family;Acetoanaerobium;genus;	Acetoanaerobium	genus	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiales_Incertae Sedis XI;family;unclassified_Clostridiales_Incertae Sedis XI;	Unclassified Clostridiales Incertae Sedis XI	family	2	0,002
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; unclassified_Clostridiales;	Unclassified Clostridiales	order	88	0,100
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;unclassified_Clostridia;	Unclassified Clostridia	class	9	0,010
Bacilli		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order; Carnobacteriaceae;family;Atopostipes;genus;	Atopostipes	genus	2	0,002
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order; Carnobacteriaceae;family;unclassified_Carnobacteriaceae;	Unclassified Carnobacteriaceae	family	157	0,179
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Aerococcaceae;family; Facklamia;genus;	Facklamia	genus	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order; unclassified_Lactobacillales;	Unclassified Lactobacillales	order	13	0,015
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Bacillales;order; unclassified_Bacillales;	Unclassified Bacillales	order	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;unclassified_Bacilli;	Unclassified Bacilli	class	3	0,003
Negativicutes		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Negativicutes;class;Selenomonadales;order; Veillonellaceae;family;Anaerosinus;genus;	Anaerosinus	genus	2	0,002
Root;rootrank;Bacteria;domain;Firmicutes;phylum;unclassified_Firmicutes;	Unclassified Firmicutes		13905	15,818
Actinobacteria		phylum		
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Actinomycineae;suborder; Actinomycetaceae;family;unclassified_Actinomycetaceae;	Unclassified Actinomycetaceae	family	2653	3,018
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Propionibacterineae;suborder;Propionibacteriaceae;family; unclassified_Propionibacteriaceae;	Unclassified Propionibacteriaceae	suborder	6	0,007
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Micrococcineae;suborder; Micrococcaceae;family;Micrococcus;genus;	Micrococcus	genus	2	0,002
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;unclassified_Actinomycetales;	Unclassified Actinomycetales	order	254	0,289
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; unclassified_Actinobacteridae;	Unclassified Actinobacteridae	subclass	2	0,002
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class; unclassified_Actinobacteria;	Unclassified Actinobacteria	class	1	0,001
Archaea	Archaea	domain		
Euryarchaeota		phylum		
Root;rootrank;Archaea;domain;"Euryarchaeota";phylum;"Methanomicrobia";class;Methanosarcinales;order; Methanotrichaceae;family;Methanothrix;genus;	Methanothrix	genus	1	0,001
Root;rootrank;Archaea;domain;"Euryarchaeota";phylum;Methanobacteria;class;Methanobacteriales;order; Methanobacteriaceae;family;Methanobacterium;genus;	Methanobacterium	genus	1	0,001
Total			87904	100

Table A3 - Relative abundance and taxonomic characterization of the microorganisms present in the reactor B (50% of refinery wastewater + 50% of synthetic medium + lactate) of the enrichment assay.

Lineage	Name	Rank	Nº sequences	Relative abundance (%)
Bacteria			84756	
Root;rootrank;Bacteria;domain;unclassified_Bacteria;	Unclassified Bacteria	domain	668	0,788
Proteobacteria		phylum		
Betaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order;Comamonadaceae;family;Diaphorobacter;genus;	Diaphorobacter	genus	42053	49,616
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order;Comamonadaceae;family;Delftia;genus;	Delftia	genus	2	0,002
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order;Comamonadaceae;family;unclassified_Comamonadaceae;	Unclassified Comamonadaceae	family	1253	1,478
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order;Alcaligenaceae;family;Pusillimonas;genus;	Pusillimonas	genus	2	0,002
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order;Rhodocyclaceae;family;Thauera;genus;	Thauera	genus	3	0,004
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order;unclassified_Burkholderiales;	Unclassified Burkholderiales	order	17	0,020
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;unclassified_Betaproteobacteria;	Unclassified Betaproteobacteria	class	4	0,005
Alphaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order;Rhodobacteraceae;family;Paracoccus;genus;	Paracoccus	genus	20	0,024
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order;Rhodobacteraceae;family;unclassified_Rhodobacteraceae;	Unclassified Rhodobacteraceae	family	12093	14,268
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order;Caulobacteraceae;family;Brevundimonas;genus;	Brevundimonas	genus	89	0,105
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order;Caulobacteraceae;family;unclassified_Caulobacteraceae;	Unclassified Caulobacteraceae	order	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;unclassified_Alphaproteobacteria;	Unclassified Alphaproteobacteria	class	528	0,623
Gammaproteobacteria		class		0,000
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order;Pseudomonadaceae;family;Pseudomonas;genus;	Pseudomonas	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order;Pseudomonadaceae;family;unclassified_Pseudomonadaceae;	Unclassified Pseudomonadaceae	family	3	0,004
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order;Moraxellaceae;family;Acinetobacter;genus;	Acinetobacter	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order;Moraxellaceae;family;unclassified_Moraxellaceae;	Unclassified Moraxellaceae	family	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Xanthomonadales;order;Xanthomonadaceae;family;Stenotrophomonas;genus;	Stenotrophomonas	genus	8	0,009
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;unclassified_Gammaproteobacteria;	Unclassified Gammaproteobacteria	class	1079	1,273
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;unclassified_"Proteobacteria";	Unclassified "Proteobacteria"	phylum	1093	1,290
Firmicutes		phylum		
Clostridia		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiales_Incertae Sedis XI;family;Tissierella;genus;	Tissierella	genus	6901	8,142
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiales_Incertae Sedis XI;family;Sedimentibacter;genus;	Sedimentibacter	genus	34	0,040

Table A3 - Relative abundance and taxonomic characterization of the microorganisms present in the reactor B (50% of refinery wastewater + 50% of synthetic medium + lactate)of the enrichment assay (continuation).

Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiales_Incertae Sedis XI;family;unclassified_Clostridiales_Incertae Sedis XI;	Unclassified Clostridiales Incertae Sedis XI	family	1791	2,113
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiaceae 1;family;Proteiniclasticum;genus;	Proteiniclasticum	genus	56	0,066
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiaceae 1;family;unclassified_Clostridiaceae 1;	Unclassified Clostridiaceae 1	family	103	0,122
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; unclassified_Clostridiales;	Unclassified Clostridiales	order	2221	2,620
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;unclassified_Clostridia;	Unclassified Clostridia	class	100	0,118
Bacilli		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Aerococcaceae;family; Facklamia;genus;	Facklamia	genus	2	0,002
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order; Carnobacteriaceae;family;unclassified_Carnobacteriaceae;	Unclassified Carnobacteriaceae	family	2	0,002
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;unclassified_Bacilli;	Unclassified Bacilli	class	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;unclassified_Firmicutes;	Unclassified Firmicutes	phylum	13393	15,802
Actinobacteria		class		
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Actinomycineae;suborder; Actinomycetaceae;family;unclassified_Actinomycetaceae;	Unclassified Actinomycetaceae	family	1155	1,363
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Propionibacterineae;suborder;Propionibacteriaceae;family; unclassified_Propionibacteriaceae;	Unclassified Propionibacteriaceae	family	1	0,001
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class; Actinobacteridae;subclass;Actinomycetales;order;Propionibacterineae;suborder; Propionibacteriaceae;family;unclassified_Propionibacteriaceae;	Unclassified Propionibacteriaceae	family	1	0,001
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class; Actinobacteridae;subclass;Actinomycetales;order;unclassified_Actinomycetales;	Unclassified Actinomycetales	order	69	0,081
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class; Actinobacteridae;subclass;unclassified_Actinobacteridae;	Unclassified Actinobacteridae	subclass	3	0,004
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class; unclassified_Actinobacteria;	Unclassified Actinobacteria	class	3	0,004
Synergistetes		phylum		
Root;rootrank;Bacteria;domain;"Synergistetes";phylum;Synergistia;class;Synergistales;order; Synergistaceae;family;unclassified_Synergistaceae;	Unclassified Synergistaceae	family	1	0,001
Cyanobacteria/Chloroplast		phylum		
Root;rootrank;Bacteria;domain;Cyanobacteria/Chloroplast;phylum;Cyanobacteria;class; unclassified_Cyanobacteria;	Unclassified Cyanobacteria	class	1	0,001
Archaea	Archaea	domain		
Euryarchaeota		phylum		
Root;rootrank;Archaea;domain;"Euryarchaeota";phylum;Methanobacteria;class; Methanobacteriales;order;Methanobacteriaceae;family;Methanobacterium;genus;	Methanobacterium	genus	1	0,001
Total			84757	100

Table A4 - Relative abundance and taxonomic characterization of the microorganisms present in the reactor C (100% of synthetic medium + glucose) of the enrichment assay.

Lineage	Name	Rank	Nº sequences	Relative abundance (%)
Bacteria			71297	
Root;rootrank;Bacteria;domain;unclassified_Bacteria;	Unclassified Bacteria	domain	292	0,410
Proteobacteria		phylum		
Betaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Diaphorobacter;genus;	Diaphorobacter	genus	63327	88,821

Table A4 - Relative abundance and taxonomic characterization of the microorganisms present in the reactor C (100% of synthetic medium + glucose) of the enrichment assay (continuation).

Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Alcaligenaceae;family;Pusillimonas;genus;	Pusillimonas	genus	139	0,195
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Alcaligenaceae;family;Pusillimonas;genus;	Pusillimonas	genus	139	0,195
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;unclassified_Comamonadaceae;	Unclassified Comamonadaceae	family	1545	2,167
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Alcaligenaceae;family;unclassified_Alcaligenaceae;	Unclassified Alcaligenaceae	family	46	0,065
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order; Rhodocyclaceae;family;Thauera;genus;	Thauera	genus	8	0,011
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; unclassified_Burkholderiales;	Unclassified Burkholderiales	order	12	0,017
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Hydrogenophilales;order; Hydrogenophilaceae;family;Tepidiphilus;genus;	Tepidiphilus	genus	2	0,003
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class; unclassified_Betaproteobacteria;	Unclassified Betaproteobacteria	class	1	0,001
Alphaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order; Rhodobacteraceae;family;Paracoccus;genus;	Paracoccus	genus	3	0,004
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order; Rhodobacteraceae;family;unclassified_Rhodobacteraceae;	Unclassified Rhodobacteraceae	family	1591	2,232
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order; Caulobacteraceae;family;Brevundimonas;genus;	Brevundimonas	genus	1786	2,505
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order; Caulobacteraceae;family;unclassified_Caulobacteraceae;	Unclassified Caulobacteraceae	family	23	0,032
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class; unclassified_Alphaproteobacteria;	Unclassified Alphaproteobacteria	class	222	0,311
Gammaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Xanthomonadales;order; Xanthomonadaceae;family;Stenotrophomonas;genus;	Stenotrophomonas	genus	19	0,027
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Moraxellaceae;family;Acinetobacter;genus;	Acinetobacter	genus	12	0,017
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Moraxellaceae;family;unclassified_Moraxellaceae;	Unclassified Moraxellaceae	family	7	0,010
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; unclassified_Pseudomonadales;	Unclassified Pseudomonadales	order	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; unclassified_Gammaproteobacteria;	Unclassified Gammaproteobacteria	class	227	0,318
Deltaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Deltaproteobacteria;class;Desulfovibrionales;order; Desulfomicrobiaceae;family;Desulfomicrobium;genus;	Desulfomicrobium	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;unclassified_"Proteobacteria";	Unclassified "Proteobacteria"	phylum	814	1,142
Alphaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order; Rhodobacteraceae;family;Paracoccus;genus;	Paracoccus	genus	3	0,004
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order; Rhodobacteraceae;family;unclassified_Rhodobacteraceae;	Unclassified Rhodobacteraceae	family	1591	2,232
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order; Caulobacteraceae;family;Brevundimonas;genus;	Brevundimonas	genus	1786	2,505
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order; Caulobacteraceae;family;unclassified_Caulobacteraceae;	Unclassified Caulobacteraceae	family	23	0,032
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class; unclassified_Alphaproteobacteria;	Unclassified Alphaproteobacteria	class	222	0,311

Table A4 - Relative abundance and taxonomic characterization of the microorganisms present in the reactor C (100% of synthetic medium + glucose) of the enrichment assay (continuation).

Gammaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Xanthomonadales;order; Xanthomonadaceae;family;Stenotrophomonas;genus;	Stenotrophomonas	genus	19	0,027
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Moraxellaceae;family;Acinetobacter;genus;	Acinetobacter	genus	12	0,017
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Moraxellaceae;family;unclassified_Moraxellaceae;	Unclassified Moraxellaceae	family	7	0,010
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; unclassified_Pseudomonadales;	Unclassified Pseudomonadales	order	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; unclassified_Gammaproteobacteria;	Unclassified Gammaproteobacteria	class	227	0,318
Deltaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Deltaproteobacteria;class;Desulfovibrionales;order; Desulfomicrobiaceae;family;Desulfomicrobium;genus;	Desulfomicrobium	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;unclassified_"Proteobacteria";	Unclassified "Proteobacteria"	phylum	814	1,142
Firmicutes		phylum		
Clostridia		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1; family;Proteiniclasticum;genus;	Proteiniclasticum	genus	99	0,139
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1; family;unclassified_Clostridiaceae 1;	Unclassified Clostridiaceae 1	family	133	0,187
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiales_Incertae Sedis XI;family;Sedimentibacter;genus;	Sedimentibacter	genus	34	0,048
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiales_Incertae Sedis XI;family;Tissierella;genus;	Tissierella	genus	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Eubacteriaceae;family; Acetobacterium;genus;	Acetobacterium	genus	2	0,003
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; unclassified_Clostridiales;	Unclassified Clostridiales	order	7	0,010
Bacilli		class		0,000
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order; Carnobacteriaceae;family;unclassified_Carnobacteriaceae;	Unclassified Carnobacteriaceae	family	21	0,029
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order; unclassified_Lactobacillales;	Unclassified Lactobacillales	order	1	0,001
Negativicutes		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Negativicutes;class;Selenomonadales;order; Veillonellaceae;family;unclassified_Veillonellaceae;	Unclassified Veillonellaceae	family	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;unclassified_Bacilli;	Unclassified Bacilli	class	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;unclassified_Firmicutes;	Unclassified Firmicutes	phylum	429	0,602
Actinobacteria		phylum		
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Actinomycineae;suborder;Actinomycetaceae;family;unclassified_Actinomycetaceae;	Unclassified Actinomycetaceae	family	413	0,579
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;unclassified_Actinomycetales;	Unclassified Actinomycetales	order	69	0,097
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; unclassified_Actinobacteridae;	Unclassified Actinobacteridae	subclass	1	0,001
Synergistetes		phylum		
Root;rootrank;Bacteria;domain;"Synergistetes";phylum;Synergistia;class;Synergistales;order;Synergistaceae;family; unclassified_Synergistaceae;	Unclassified Synergistaceae	family	6	0,008
Thermotogae		phylum		
Root;rootrank;Bacteria;domain;"Thermotogae";phylum;Thermotogae;class;Thermotogales;order;Thermotogaceae;family; unclassified_Thermotogaceae;	Unclassified Thermotogaceae	family	1	0,001
Total			71297	100

Table A5 -Relative abundance and taxonomic characterization of the microorganisms present in the reactor D (100% of synthetic medium + lactate) of the enrichment assay.

Lineage	Name	Rank	Nº sequences	Relative abundance (%)
Bacteria			65018	
Root;rootrank;Bacteria;domain;unclassified_Bacteria;	Unclassified Bacteria	domain	586	0,901
Proteobacteria		phylum		
Betaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Diaphorobacter;genus;	Diaphorobacter	genus	35374	54,406
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;unclassified_Comamonadaceae;	Unclassified Comamonadaceae	family	953	1,466
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Alcaligenaceae;family;Pusillimonas;genus;	Pusillimonas	genus	50	0,077
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Alcaligenaceae;family;unclassified_Alcaligenaceae;	Unclassified Alcaligenaceae	family	12	0,018
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order; Rhodocyclaceae;family;Thauera;genus;	Thauera	genus	116	0,178
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order; Rhodocyclaceae;family;unclassified_Rhodocyclaceae;	Unclassified Rhodocyclaceae	family	9	0,014
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; unclassified_Burkholderiales;	Unclassified Burkholderiales	order	11	0,017
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class; unclassified_Betaproteobacteria;	Unclassified Betaproteobacteria	class	35	0,054
Alphaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order; Rhodobacteraceae;family;Paracoccus;genus;	Paracoccus	genus	13	0,020
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order; Rhodobacteraceae;family;unclassified_Rhodobacteraceae;	Unclassified Rhodobacteraceae	family	6132	9,431
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order; Caulobacteraceae;family;Brevundimonas;genus;	Brevundimonas	genus	188	0,289
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order; Caulobacteraceae;family;unclassified_Caulobacteraceae;	Unclassified Caulobacteraceae	family	1	0,002
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhizobiales;order; Methylocystaceae;family;Methylosinus;genus;	Methylosinus	genus	1	0,002
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class; unclassified_Alphaproteobacteria;	Unclassified Alphaproteobacteria	class	325	0,500
Gammaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Xanthomonadales;order; Xanthomonadaceae;family;Stenotrophomonas;genus;	Stenotrophomonas	genus	8	0,012
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; Pseudomonadales;order;Moraxellaceae;family;Acinetobacter;genus;	Acinetobacter	genus	1	0,002
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; Pseudomonadales;order;Moraxellaceae;family;unclassified_Moraxellaceae;	Unclassified Moraxellaceae	family	5	0,008
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; Pseudomonadales;order;Pseudomonadaceae;family;unclassified_Pseudomonadaceae;	Unclassified Pseudomonadaceae	family	1	0,002
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; unclassified_Gammaproteobacteria;	Unclassified Gammaproteobacteria	class	291	0,448
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;unclassified_"Proteobacteria";	Unclassified "Proteobacteria"	phylum	799	1,229
Firmicutes		phylum		
Clostridia		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiales_Incertae Sedis XI;family;Sedimentibacter;genus;	Sedimentibacter	genus	17096	26,294

Table A5 - Relative abundance and taxonomic characterization of the microorganisms present in the reactor D (100% of synthetic medium + lactate) of the enrichment assay (continuation).

Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiales_Incertae Sedis XI;family;Sedimentibacter;genus;	Sedimentibacter	genus	17096	26,294
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiales_Incertae Sedis XI;family;Tissierella;genus;	Tissierella	genus	471	0,724
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiales_Incertae Sedis XI;family;unclassified_Clostridiales_Incertae Sedis XI;	Unclassified Clostridiales Incertae Sedis XI	family	145	0,223
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiaceae 1;family;Proteiniclasticum;genus;	Proteiniclasticum	genus	49	0,075
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; Clostridiaceae 1; family; unclassified_Clostridiaceae 1;	Unclassified Clostridiaceae 1	family	83	0,128
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;unclassified_Clostridiales;	Unclassified Clostridiales	order	354	0,544
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;unclassified_Clostridia;	Unclassified Clostridia	class	35	0,054
Bacilli	Bacilli	class		0,000
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Carnobacteriaceae;family; unclassified_Carnobacteriaceae;	Unclassified Carnobacteriaceae	family	5	0,008
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;unclassified_Bacilli;	Unclassified Bacilli	class	1	0,002
Root;rootrank;Bacteria;domain;Firmicutes;phylum;unclassified_Firmicutes;	Unclassified Firmicutes	phylum	1679	2,582
Actinobacteria		phylum		
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Actinomycineae;suborder; Actinomycetaceae;family;unclassified_Actinomycetaceae;	Unclassified_Actinomycetaceae	family	168	0,258
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;unclassified_Actinomycetales;	Unclassified Actinomycetales	order	17	0,026
Thermotogae		phylum		
Root;rootrank;Bacteria;domain;"Thermotogae";phylum;Thermotogae;class;Thermotogales;order; Thermotogaceae;family;unclassified_Thermotogaceae;	Unclassified Thermotogaceae	family	1	0,002
Deinococcus-Thermus	Deinococcus-Thermus	phylum		
Root;rootrank;Bacteria;domain;"Deinococcus – Thermus";phylum;Deinococci;class; Thermales;order;Thermaceae;family;Thermus;genus;	Thermus	genus	1	0,002
Synergistetes		phylum		
Root;rootrank;Bacteria;domain;"Synergistetes";phylum;Synergistia;class;Synergistales;order; Synergistaceae;family;unclassified_Synergistaceae;	Unclassified Synergistaceae	family	2	0,003
Total			65018	100

Table A1 - Relative abundance and taxonomic characterization of the microorganisms present at time 0 of biodegradation assay.

Lineage	Name	Rank	Nº sequences	Relative abundance (%)
Bacteria			106533	
Root;rootrank;Bacteria;domain;unclassified_Bacteria;	Unclassified Bacteria	domain	3645	3,408
Proteobacteria		phylum		
Betaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order; Rhodocyclaceae;family;Thauera;genus;	Thauera	genus	11209	10,481
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order; Rhodocyclaceae;family;unclassified_Rhodocyclaceae;	Unclassified Rhodocyclaceae	family	6460	6,041
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Diaphorobacter;genus;	Diaphorobacter	genus	25562	23,903
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Delftia;genus;	Delftia	genus	26	0,024

Table A6 – Relative abundance and taxonomic characterization of the microorganisms present at time 0 of biodegradation assay (continuation).

Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Acidovorax;genus;	Acidovorax	genus	362	0,339
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Alicycliphilus;genus;	Alicycliphilus	genus	3	0,003
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Brachymonas;genus;	Brachymonas	genus	3	0,003
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;unclassified_Comamonadaceae;	Unclassified Comamonadaceae	family	8943	8,362
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; unclassified_Burkholderiales;	Unclassified Burkholderiales	order	756	0,707
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class; unclassified_Betaproteobacteria;	Unclassified Betaproteobacteria	class	37125	34,715
Alphaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order; Rhodobacteraceae;family;Paracoccus;genus;	Paracoccus	genus	40	0,037
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order; Rhodobacteraceae;family;unclassified_Rhodobacteraceae;	Unclassified Rhodobacteraceae	family	3265	3,053
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order; Caulobacteraceae;family;Brevundimonas;genus;	Brevundimonas	genus	9	0,008
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class; unclassified_Alphaproteobacteria;	Unclassified Alphaproteobacteria	class	211	0,197
Gammaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Moraxellaceae;family;Acinetobacter;genus;	Acinetobacter	genus	49	0,046
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Moraxellaceae;family;Psychrobacter;genus;	Psychrobacter	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Moraxellaceae;family;unclassified_Moraxellaceae;	Unclassified Moraxellaceae	family	2	0,002
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Pseudomonadaceae;family;Pseudomonas;genus;	Pseudomonas	genus	2	0,002
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; unclassified_Gammaproteobacteria;	Unclassified Gammaproteobacteria	class	50	0,047
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;unclassified_"Proteobacteria";	Unclassified "Proteobacteria"	phylum	3503	3,276
Firmicutes		phylum		
Clostridia		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1;family; Proteiniclasticum;genus;	Proteiniclasticum	genus	2414	2,257
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1;family; Clostridium sensu stricto;genus;	Clostridium sensu stricto	genus	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1;family; unclassified_Clostridiaceae 1;	Unclassified Clostridiaceae 1	family	285	0,266
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; unclassified_Clostridiales;	Unclassified Clostridiales	class	25	0,023
Bacilli		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Streptococcaceae;family; Streptococcus;genus;	Streptococcus	genus	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Leuconostocaceae;family; Leuconostoc;genus;	Leuconostoc	genus	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Enterococcaceae;family; Bavariicoccus;genus;	Bavariicoccus	genus	12	0,011
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Enterococcaceae;family; unclassified_Enterococcaceae;	Unclassified Enterococcaceae	family	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Aerococcaceae;family; Ignavigranum;genus;	Ignavigranum	genus	12	0,011
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Aerococcaceae;family; unclassified_Aerococcaceae;	Unclassified Aerococcaceae	family	252	0,236
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order; unclassified_Lactobacillales;	Unclassified Lactobacillales	order	179	0,167

Table A6 – Relative abundance and taxonomic characterization of the microorganisms present at time 0 of biodegradation assay (continuation).

Root;rootrank;Bacteria;domain;Firmicutes;phylum;unclassified_Firmicutes;	Unclassified Firmicutes		1664	1,556
Actinobacteria		phylum		
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Actinomycineae;suborder;Actinomycetaceae;family;unclassified_Actinomycetaceae;	Unclassified Actinomycetaceae	family	259	0,242
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Propionibacterineae;suborder;Nocardioidaceae;family;Nocardioides;genus;	Nocardioides	genus	1	0,001
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Micrococcineae;suborder;Cellulomonadaceae;family;unclassified_Cellulomonadaceae;	Unclassified Cellulomonadaceae	family	1	0,001
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Micrococcineae;suborder;unclassified_Micrococcineae;	Unclassified Micrococcineae	suborder	1	0,001
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;unclassified_Actinomycetales;	Unclassified Actinomycetales	order	148	0,138
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; unclassified_Actinobacteridae;	Unclassified Actinobacteridae	subclass	8	0,007
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;unclassified_Actinobacteria;	Unclassified Actinobacteria	class	7	0,007
unclassified_ Root			409	0,00382
Total			106942	100

Table A7 – Relative abundance and taxonomic characterization of the microorganisms present at reactor A (Inoculum + Acenaphthene + Lactate) of biodegradation assay.

Lineage	Name	Rank	Nº sequences	Relative abundance (%)
Bacteria			119845	
Root;rootrank;Bacteria;domain;unclassified_Bacteria;	Unclassified_Bacteria	domain	7707	6,380
Proteobacteria		phylum		
Betaproteobacteria	Betaproteobacteria	class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Brachymonas;genus;	Brachymonas	genus	841	0,696
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Diaphorobacter;genus;	Diaphorobacter	genus	24471	20,258
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Alicycliphilus;genus;	Alicycliphilus	genus	4	0,003
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Acidovorax;genus;	Acidovorax	genus	173	0,143
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Delftia;genus;	Delftia	genus	21	0,017
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;unclassified_Comamonadaceae;	Unclassified Comamonadaceae	family	6747	5,585
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order; Rhodocyclaceae;family;Thauera;genus;	Thauera	genus	9421	7,799
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order; Rhodocyclaceae;family;unclassified_Rhodocyclaceae;	Unclassified Rhodocyclaceae	family	4230	3,502
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; unclassified_Burkholderiales;	Unclassified Burkholderiales	order	680	0,563
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class; unclassified_Betaproteobacteria;	Unclassified Betaproteobacteria	class	34515	28,573
Alphaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order; Caulobacteraceae;family;Brevundimonas;genus;	Brevundimonas	genus	12685	10,501
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order; Caulobacteraceae;family;unclassified_Caulobacteraceae;	Unclassified Caulobacteraceae	family	229	0,190

Table A7 –Relative abundance and taxonomic characterization of the microorganisms present at reactor A (Inoculum + Acenaphthene + Lactate) of biodegradation assay (continuation).

Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order; unclassified_Caulobacterales;	Unclassified Caulobacterales	order	125	0,103
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order; Rhodobacteraceae;family;Paracoccus;genus;	Paracoccus	genus	43	0,036
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order; Rhodobacteraceae;family;unclassified_Rhodobacteraceae;	Unclassified Rhodobacteraceae	family	2166	1,793
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class; unclassified_Alphaproteobacteria;	Unclassified Alphaproteobacteria	class	1316	1,089
Gammaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Moraxellaceae;family;Acinetobacter;genus;	Acinetobacter	genus	35	0,029
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Moraxellaceae;family;Enhydrobacter;genus;	Enhydrobacter	genus	2	0,002
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Pseudomonadaceae;family;Pseudomonas;genus;	Pseudomonas	genus	3	0,002
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; unclassified_Gammaproteobacteria;	Unclassified Gammaproteobacteria	class	7	0,006
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;unclassified_"Proteobacteria";	Unclassified "Proteobacteria"	phylum	4100	3,394
Bacteroidetes		phylum		
Bacteroidia		class		
Root;rootrank;Bacteria;domain;"Bacteroidetes";phylum;"Bacteroidia";class;"Bacteroidales";order;" Porphyromonadaceae";family;Proteiniphilum;genus;	Proteiniphilum	genus	1419	1,175
Root;rootrank;Bacteria;domain;"Bacteroidetes";phylum;"Bacteroidia";class;"Bacteroidales";order; "Porphyromonadaceae";family;unclassified_"Porphyromonadaceae";	unclassified "Porphyromonadaceae"	family	224	0,185
Root;rootrank;Bacteria;domain;"Bacteroidetes";phylum;"Bacteroidia";class;"Bacteroidales"; order;unclassified_"Bacteroidales";	unclassified "Bacteroidales"	order	35	0,029
Root;rootrank;Bacteria;domain;"Bacteroidetes";phylum;unclassified_"Bacteroidetes";	unclassified "Bacteroidetes"	phylum	68	0,056
Firmicutes		phylum		
Clostridia		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1;family; Proteiniclasticum;genus;	Proteiniclasticum	genus	3339	2,764
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1;family; Clostridium sensu stricto;genus;	Clostridium sensu stricto	genus	4	0,003
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1;family; unclassified_Clostridiaceae 1;	unclassified Clostridiaceae 1	family	416	0,344
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order; unclassified_Clostridiales;	unclassified Clostridiales	order	44	0,036
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;unclassified_Clostridia;	unclassified Clostridia	class	1	0,001
Bacilli		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order; Aerococcaceae;family;Ignavigranum;genus;	Ignavigranum	genus	7	0,006
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order; Aerococcaceae;family;unclassified_Aerococcaceae;	Unclassified Aerococcaceae	family	278	0,230
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order; Enterococcaceae;family;Bavariicoccus;genus;	Bavariicoccus	genus	15	0,012
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order; Enterococcaceae;family;unclassified_Enterococcaceae;	Unclassified Enterococcaceae	family	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order; unclassified_Lactobacillales;	Unclassified Lactobacillales	order	184	0,152
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;unclassified_Bacilli;	Unclassified Bacilli	class	63	0,052
Root;rootrank;Bacteria;domain;Firmicutes;phylum;unclassified_Firmicutes;	Unclassified Firmicutes	phylum	3361	2,782
Actinobacteria		phylum		
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Micrococcineae;suborder;Cellulomonadaceae;family;Cellulomonas;genus;	Cellulomonas	genus	5	0,004

Table A7 – Relative abundance and taxonomic characterization of the microorganisms present at reactor A (Inoculum + Acenaphthene + Lactate) of biodegradation assay (continuation).

Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Micrococcineae;suborder;Cellulomonadaceae;family;unclassified_Cellulomonadaceae; unclassified_Cellulomonadaceae;	Unclassified Cellulomonadaceae	family	33	0,027
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Micrococcineae;suborder;unclassified_Micrococcineae;	Unclassified Micrococcineae	suborder	47	0,039
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Propionibacterineae;suborder;Nocardioidaceae;family;Nocardioides;genus;	Nocardioides	genus	1	0,001
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;Actinomycineae;suborder;Actinomycetaceae;family;unclassified_Actinomycetaceae;	Unclassified Actinomycetaceae	family	214	0,177
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; Actinomycetales;order;unclassified_Actinomycetales;	Unclassified Actinomycetales	order	554	0,459
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; unclassified_Actinobacteridae;	Unclassified Actinobacteridae	subclass	8	0,007
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;unclassified_Actinobacteria;	Unclassified Actinobacteria	class	2	0,002
Fusobacteria		phylum		
Fusobacteriia		class		
Root;rootrank;Bacteria;domain;"Fusobacteria";phylum;Fusobacteriia;class;"Fusobacteriales";order; "Leptotrichiaceae";family;unclassified_"Leptotrichiaceae";	unclassified "Leptotrichiaceae"		1	0,001
unclassified_ Root			951	0,787
Total			120796	100

Table A2 – Relative abundance and taxonomic characterization of the microorganisms present at reactor C (Inoculum + Acenaphthene) of biodegradation assay.

Lineage	Name	Rank	Nº sequences	Relative abundance (%)
Bacteria			116356	
Root;rootrank;Bacteria;domain;unclassified_Bacteria;	Unclassified Bacteria	Domain	5895	5,062
Proteobacteria		phylum		
Gammaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Moraxellaceae;family;Acinetobacter;genus;	Acinetobacter	genus	16415	14,096
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Moraxellaceae;family;Alkanindiges;genus;	Alkanindiges	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; Moraxellaceae;family;unclassified_Moraxellaceae;	Unclassified Moraxellaceae	family	1502	1,290
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Legionellales;order; Legionellaceae;family;Legionella;genus;	Legionella	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class;Pseudomonadales;order; unclassified_Pseudomonadales;	Unclassified Pseudomonadales	order	570	0,489
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Gammaproteobacteria;class; unclassified_Gammaproteobacteria;	Unclassified Gammaproteobacteria	class	35516	30,499
Betaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Diaphorobacter;genus;	Diaphorobacter	genus	14058	12,072
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Acidovorax;genus;	Acidovorax	genus	137	0,118
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Delftia;genus;	Delftia	genus	8	0,007
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Alicycliphilus;genus;	Alicycliphilus	genus	3	0,003
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order; Comamonadaceae;family;Brachymonas;genus;	Brachymonas	genus	15	0,013

Table A8 – Relative abundance and taxonomic characterization of the microorganisms present at reactor C (Inoculum + Acenaphthene) of biodegradation assay (continuation).

Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order;Comamonadaceae;family;Acidovorax;genus;	Acidovorax	genus	137	0,118
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order;Comamonadaceae;family;Delftia;genus;	Delftia	genus	8	0,007
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order;Comamonadaceae;family;Alicycliphilus;genus;	Alicycliphilus	genus	3	0,003
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order;Comamonadaceae;family;Brachymonas;genus;	Brachymonas	genus	15	0,013
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order;Comamonadaceae;family;unclassified_Comamonadaceae;	Unclassified Comamonadaceae	family	4951	4,252
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Burkholderiales;order;unclassified_Burkholderiales;	Unclassified Burkholderiales	order	320	0,275
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order;Rhodocyclaceae;family;Thauera;genus;	Thauera	genus	2120	1,821
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order;Rhodocyclaceae;family;Dechloromonas;genus;	Dechloromonas	genus	1	0,001
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class;Rhodocyclales;order;Rhodocyclaceae;family;unclassified_Rhodocyclaceae;	Unclassified Rhodocyclaceae	family	1038	0,891
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Betaproteobacteria;class; unclassified_Betaproteobacteria;	Unclassified Betaproteobacteria	class	9143	7,852
Alphaproteobacteria		class		
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order;Rhodobacteraceae;family;Paracoccus;genus;	Paracoccus	genus	11	0,009
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Rhodobacterales;order;Rhodobacteraceae;family;unclassified_Rhodobacteraceae;	Unclassified Rhodobacteraceae	family	1105	0,949
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order;Caulobacteraceae;family;Brevundimonas;genus;	Brevundimonas	genus	173	0,149
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class;Caulobacterales;order;Caulobacteraceae;family;unclassified_Caulobacteraceae;	Unclassified Caulobacteraceae	order	3	0,003
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;Alphaproteobacteria;class; unclassified_Alphaproteobacteria;	Unclassified Alphaproteobacteria	class	89	0,076
Root;rootrank;Bacteria;domain;"Proteobacteria";phylum;unclassified_"Proteobacteria";	Unclassified "Proteobacteria"	phylum	5106	4,385
Firmicutes		phylum		
Clostridia		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1;family;Proteiniclasticum;genus;	Proteiniclasticum	genus	14383	12,351
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1;family;Clostridium sensu stricto;genus;	Clostridium sensu stricto	genus	151	0,130
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;Clostridiaceae 1;family;unclassified_Clostridiaceae 1;	Unclassified Clostridiaceae 1	family	1272	1,092
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;Clostridiales;order;unclassified_Clostridiales;	Unclassified Clostridiales	order	168	0,144
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Clostridia;class;unclassified_Clostridia;	Unclassified Clostridia	class	16	0,014
Bacilli		class		
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Streptococcaceae;family;unclassified_Streptococcaceae;	Unclassified Streptococcaceae	family	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Aerococcaceae;family;Ignavigranum;genus;	Ignavigranum	genus	4	0,003
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Aerococcaceae;family;unclassified_Aerococcaceae;	Unclassified Aerococcaceae	family	32	0,027
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;Enterococcaceae;family;Bavariicoccus;genus;	Bavariicoccus	genus	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Lactobacillales;order;unclassified_Lactobacillales;	Unclassified Lactobacillales	order	36	0,031

Table A8 – Relative abundance and taxonomic characterization of the microorganisms present at reactor C (Inoculum + Acenaphthene) of biodegradation assay (continuation).

Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;Bacillales;order;unclassified_Bacillales;	Unclassified Bacillales	order	1	0,001
Root;rootrank;Bacteria;domain;Firmicutes;phylum;Bacilli;class;unclassified_Bacilli;	Unclassified Bacilli	class	21	0,018
Root;rootrank;Bacteria;domain;Firmicutes;phylum;unclassified_Firmicutes;	Unclassified Firmicutes	phylum	945	0,812
Actinobacteria		phylum		
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass;Actinomycetales;order; Actinomycineae;suborder;Actinomycetaceae;family;unclassified_Actinomycetaceae;	Unclassified Actinomycetaceae	family	72	0,062
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass;Actinomycetales;order; Micrococcineae;suborder;Cellulomonadaceae;family;Cellulomonas;genus;	Cellulomonas	genus	2	0,002
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass;Actinomycetales;order; Micrococcineae;suborder;Cellulomonadaceae;family;unclassified_Cellulomonadaceae;	Unclassified Cellulomonadaceae	family	2	0,002
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass;Actinomycetales;order; Micrococcineae;suborder;unclassified_Micrococcineae;	Unclassified Micrococcineae	suborder	9	0,008
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass;Actinomycetales;order; unclassified_Actinomycetales;	Unclassified Actinomycetales	order	60	0,052
Root;rootrank;Bacteria;domain;"Actinobacteria";phylum;Actinobacteria;class;Actinobacteridae;subclass; unclassified_Actinobacteridae;	Unclassified Actinobacteridae	subclass	2	0,002
Bacteroidetes		phylum		
Root;rootrank;Bacteria;domain;"Bacteroidetes";phylum;"Bacteroidia";class;"Bacteroidales";order; "Porphyromonadaceae";family;Proteiniphilum;genus;	Proteiniphilum	genus	782	0,672
Root;rootrank;Bacteria;domain;"Bacteroidetes";phylum;"Bacteroidia";class;"Bacteroidales";order; "Porphyromonadaceae";family;unclassified_"Porphyromonadaceae";	Unclassified "Porphyromonadaceae"	family	143	0,123
Root;rootrank;Bacteria;domain;"Bacteroidetes";phylum;"Bacteroidia";class;"Bacteroidales";order; unclassified_"Bacteroidales";	Unclassified "Bacteroidales"	order	28	0,024
Root;rootrank;Bacteria;domain;"Bacteroidetes";phylum;unclassified_"Bacteroidetes";	Unclassified "Bacteroidetes"	phylum	44	0,038
unclassified_Root			92	0,079
Total			116448	100